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STUDIES UPON THE SECRETION OF ORAL AND PHARYNGEAL MUCUS[1]

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The oral and pharyngeal mucous glands are known to receive cranial autonomic and sympathetic secretory fibers (1a), and to be excited by injections of suprarenal extracts (2) and of pilocarpin (3). The reflex secretion of certain groups of these glands was observed by Kehrer (4) and by Heyman (5a) in the dog, and by Rossbach (6) in man. Colin (5b) reported a continuous activity of the oral mucous glands in ruminants, but Schäfer (1b) believed this secretion to be excited reflexly. Bidder and Schmidt (7) were never able to collect more than 2 grams of oral mucus per hour in dogs, and stated that Jacubowitsch obtained only slightly more than 2 grams from the mouth in 52 minutes. The only quantitative measurements of oral mucous secretion are those of Heyman, who measured the responses of the orbital gland (posterior molar glands) of the dog to food and chemical stimuli, and compared the volumes of orbital, submaxillary, and sublingual glandular secretion induced by mechanical stimulation of the mouth (5a).

In view of the scanty literature on this subject, we undertook to compare the secretory responses of oral and pharyngeal mucous glands with those of the salivary glands.

METHODS. In some experiments the oral and pharyngeal mucous membranes were directly observed in dogs and human subjects. In others collections of mucus were made by means of esophageal fistulae on 7 dogs in which the salivary glands had first been extirpated or the salivary ducts tied. The orbital glands also were excised in dogs 1 to 5, but were present in dogs 6 and 7. Dog 6 had an esophageal fistula and bilateral submaxillary and parotid duct fistulae to permit the simultaneous collection of

[1] This work has been conducted in part under a grant from the Christine Breon Fund for Medical Research in the University of California Medical School.

oral mucus and saliva. Dog 7 had esophageal and right submaxillary duct fistulae.

Estimations of the secretory responses of the mucous glands were made by placing a weighed quantity of the stimulating material in the mouth and, after a definite time interval, collecting the combined secretion and stimulating substance from the esophageal fistula. The collection was facilitated by manipulating the fistula, causing the dog to swallow. In the dogs with multiple fistulae the secretion of each gland was collected separately on pledgets of cotton, and weighed.

The dogs were fed through a $\frac{3}{4}$-inch brass cannula in the stomach and, when necessary, received injections of physiological salt solution, to ensure an adequate fluid intake.

Innervation of the mucous glands. Montgomery (8a) has described the parasympathetic innervation of the oral and pharyngeal mucous glands of the dog through the terminal sensory branches of the fifth, ninth, and tenth cranial nerves and the chorda tympani nerve as determined by the secretory response to faradic stimulation of these branches. In the light of additional observations we believe, now, that this description is correct with the exception that the orbital gland is supplied by the buccinator nerve alone and does not receive fibers from the maxillary division of the fifth cranial nerve.

Drugs. Continuing the experiments begun by Montgomery (8b), we noted the secretory responses of the oral and pharyngeal mucous glands to various drugs.

Injections of pilocarpin sulfate, physostigmin, and acetylcholin induced secretion from all of the oral and pharyngeal mucous glands in anesthetized dogs. Montgomery (8b) gives quantitative determinations, on 3 dogs having neither salivary nor orbital glands, of the mucus collected from esophageal fistulae during successive 5-minute periods following the intravenous injection of 0.2, 0.3, and 0.5 mgm. per kilogram of pilocarpin. The quantities of saliva and mucus secreted by the dog with multiple fistulae (no. 6) in response to 0.2 mgm. per kilogram of pilocarpin are compared in table 5.

Atropin sulfate antagonized the effects of these drugs and abolished the mucous secretion reflexly induced in unanesthetized dogs, but did not eliminate the secretory response to faradic stimulation of the cervical sympathetic trunk, nor to intravenous injections of epinephrin.

Minute quantities of mucus appeared after the intravenous injection of epinephrin, in single or repeated doses of 0.25 to 1.0 cc. of a 1/10,000 solution, in only 3 of the 6 dogs which we observed (ether or sodium amytal anesthesia), although faradic stimulation of the cervical sympathetic trunk in the neck in these animals excited the usual unilateral secretion of mucus. In two of the responsive dogs, the effect of epinephrin persisted after the injection of from 2 to 4 mgm. of atropin sulfate.

Making 5-minute collections on 3 of the dogs with esophageal fistulae, we observed, in from 2 to 4 minutes after the subcutaneous injection of 15 mgm. of morphin sulfate, an increased production of mucus, coinciding with the appearance of retching and licking. The rate of secretion returned to the resting level in from 30 to 40 minutes after the injection, and from that time on was subnormal. During the period of depression there was a diminished response to reflex stimulation. For example, the normal quantity of secretion elicited by giving 1 cc. of a 25 per cent solution of sodium chloride orally in dog 4 was 4.05 grams (the average of 3 determinations), while 45 minutes after the injection of a 15 mgm. dose of morphin it was 2.58 grams (the average of 3 determinations).

The reflex stimulation of oral mucous secretion. Confirming and extending the observations of other investigators, we found that the oral mucous glands can be reflexly excited by a variety of stimuli. We observed the lower lip and cheek in 5 normal dogs, and the upper and lower lips and the soft palate in 4 human subjects. During a control period of from 1 to 3 minutes the exposed membranes often remained dry, but occasionally produced a few drops of secretion. In man drops of secretion appeared within a few seconds when one-half normal hydrochloric acid, normal acetic acid, crystalline sucrose, sodium chloride, or magnesium sulfate were placed on the tongue. The inhaled vapors of ether, acetic acid, or ammonium hydroxide, as well as firm pressure upon the hard palate or the base of the tongue induced copious secretion. In dogs, effective stimuli were swallowing and licking movements, rubbing of the tongue and palate, whining, retching excited by gastric distention, and the inhibition of the impulse to defecate. Conditioned reflex responses to food and taste stimuli were easily established.

Experiments upon dogs convinced us that the secretion of oral and pharyngeal mucus does not proceed continuously in the absence of reflex stimulation, for we observed the everted lower lips of unanesthetized, resting dogs, noting no secretion during 30 or more minutes, although at the end of this time the application of a variety of reflex stimuli promptly induced a copious flow of mucus.

We could not directly observe other groups of glands without reflexly inducing secretion. We therefore resorted to collecting the combined secretion of the oral and pharyngeal glands after varying intervals of rest, by means of a minimal mechanical stimulation of an esophageal fistula, to show that in dogs the activity of the glands is not continuous. In the presence of a continuous secretion one would expect the quantities of mucus obtained at each collection to increase with a prolongation of the interval between successive stimulations. This proved not to be the case. The amounts of secretion collected varied roughly within the same limits, regardless of the interval of rest, provided that the dog was completely relaxed. The data in table 1 show how uniform were the quantities of

mucus secreted for all durations of the interval of rest by 2 particularly docile and well trained animals. Five other dogs showed greater ranges of variation, as well as a few marked discrepancies in the data, which are not surprising in view of the possibility of psychic stimulation or of spontaneous licking and swallowing.

These experiments appear to us to justify our conclusion that in the dog the oral and pharyngeal mucus is produced solely in reflex response to a variety of stimuli. Under normal waking conditions it is entirely possible that these reflexes may keep the mucous glands almost continuously active.

Quantitative studies upon the reflex production of oral and pharyngeal mucus. Mechanical stimulation. In table 1 we have shown the responses of the mucous glands of dogs to simple swallowing. Vigorous and pro-

TABLE 1

The secretion of oral and pharyngeal mucus in response to minimal mechanical stimulation of the esophagus after varying intervals of rest

	INTERVALS BETWEEN COLLECTIONS	NUMBER OF COLLECTIONS	SECRETION		
			Average	Maximum	Minimum
	minutes		*grams*	*grams*	*grams*
Salivary secretion excluded from mouth:					
Dog 6	1	10	0.21	0.36	0.10
	5	10	0.20	0.42	0.10
	10	10	0.21	0.37	0.04
	15	5	0.19	0.38	0.10
	30	5	0.25	0.40	0.13
	60	1	0.28		
Dog 7	1	10	0.14	0.23	0.03
	5	10	0.14	0.30	0.04
	10	10	0.12	0.24	0.04
	15	5	0.11	0.20	0.04
	30	5	0.18	0.24	0.09
	60	1	0.20		

longed licking, as well as gagging, increased the flow of mucus. Attempts to administer inert materials, such as sand, to dogs were generally unsuccessful and the quantities of mucus obtained were relatively small. Distilled water at room temperature proved to be an extremely weak stimulus.

Taste and food stimuli. In an extensive series of collections on dogs with esophageal fistulae we observed that hydrochloric and acetic acids were the most powerful stimuli to the secretion of oral mucus, followed in decreasing order by sodium chloride and sucrose. Magnesium sulfate (bitter) was sometimes more effective than salt, sometimes less so. Of the foods tried, raw meat excited the mucous glands the least. Fresh bread induced much more secretion, and dry bread crumbs, dried meat

crumbs, or peptone powder were the most effective stimuli. In all instances there was much licking associated with the ingestion of the dry foods. We do not know what part of the mucus was secreted as a result of the movements of the mouth and what part in response to taste and to odor. Representative data on dog 6 are shown in table 2.

We noted some variability in the responses of a single dog to the same stimulus on different occasions, due probably to small changes in the method of administration or to psychic factors. On other occasions there was a striking uniformity of response by different animals to the same stimulus. The dogs were approximately the same size, weighing between 8 and 10 kgm. The absence of the orbital glands in some of these dogs did not noticeably alter the quantities of mucus collected. Two dogs with orbital glands consistently secreted approximately the same quantity of mucus as dogs of nearly the same weight without orbital glands.

The quantitative relationships of the oral and pharyngeal mucus and of the salivary glandular secretions. The relative quantities of the salivary and mucous secretions were determined by collecting them simultaneously from multiple fistulae in dog 6. Observations were also made upon dog 7, a dog with a right submaxillary-sublingual duct fistula and an esophageal fistula. Table 2 contains representative data selected from experiments on dog 6. The right parotid gland of this dog frequently, but not invariably, secreted considerably less than the left parotid gland, in spite of an apparently equal secretory capacity of the two glands, as shown by their almost identical responses to moderate doses of pilocarpin (see table 5). Since the submaxillary glands of the two sides showed more nearly equal responses, we do not believe that the phenomenon of homolateral reflex stimulation (5d) could have been the chief factor underlying the relative inactivity of the right parotid gland. In the following discussion the left parotid gland, rather than the right, will be considered to have had the more normal response.

Mechanical stimulation of the esophageal fistula, which was used to induce swallowing in all of these experiments, resulted in the formation of a small quantity of mucus, but little or no saliva. Distilled water slightly increased the secretion of mucus, but induced no significant flow of saliva.

Taste and food stimuli. Our observations confirmed the conclusions of other investigators in showing that the submaxillary and parotid glands secrete at different rates, depending upon the nature of the stimulating substances (5e). They further demonstrated that the mucous glands also have characteristic responses. Moreover, by using rather weak stimuli we observed threshold responses and noted that the relative quantities of secretion produced by the different glands changed directly with the strength of the stimulus. The mucous glands showed a lower threshold than the salivary glands for all stimuli except possibly acid. The sub-

TABLE 2

The responses of the salivary glands and of the oral and pharyngeal mucous glands to mechanical, chemical, and food stimuli

Dog 6 (F., Wt. 10.3 kgm.)

STIMULATING MATERIAL	GIVEN IN 5 MINUTES	AVERAGE SECRETION (5 MINUTES)					
		Number of experiment	Mucous	Submaxillary		Parotid	
				Right	Left	Right	Left
	parts		*grams*	*grams*	*grams*	*grams*	*grams*
Swallowing		20	0.19	0.01	0.01	0.003	0.004
Distilled water 1 cc	1	4	0.64	0.03	0.05	0.02	0.03
Sucrose:							
25% 1 cc	1	8	0.98	0.05	0.06	0.04	0.05
50% 1 cc	1	2	0.93	0.05	0.05	0.06	0.11
50% 1 cc	10	2	1.92	0.08	0.09	0.16	0.13
50% 3 cc	Contin.†	2	1.77	0.08	0.08	0.05	0.02
Crystalline, 0.5 gm	1	3	1.17	0.63	0.70	0.25	0.46
HCl:							
N/10 1 cc	1	8	1.38	0.32	0.44	0.55	0.92
N/2 1 cc	1	2	2.46	2.59	2.56	1.72	2.40
N/10 3 cc	Contin.	3	3.17	3.53	3.19	3.85	3.90
*N/10 2 cc	See footnote	8	2.16	2.16	2.01	1.59	2.13
N/2 1 cc	10						
NaCl:							
5% 1 cc	1	4	1.87	0.50	0.33	0.29	0.41
10% 1 cc	1	3	2.43	1.63	1.69	0.92	1.31
20% 1 cc	1						
25% 1 cc	1	11	2.77	2.85	3.21	0.95	1.62
Crystalline, 0.25 gm	1	3	3.48	6.93	5.18	2.59	3.13
NaCl:							
5% 3 cc	Contin.	3	2.48	0.14	0.10	0.46	0.55
10% 3 cc	Contin.	2	4.30	2.81	3.01	2.60	2.84
20% 1 cc	Contin.						
25% 1 cc	Contin.	2	3.09	2.30	2.03	2.55	2.58
25% 2 cc	Contin.	1	5.85	5.40	5.92	3.70	4.36
$MgSO_4$:							
5% 1 cc	1	3	1.34	0.18	0.15	0.39	0.44
12.5% 1 cc	1	5	1.99	1.03	1.74	0.47	1.06
25% 1 cc	1	5	2.59	3.68	4.58	1.98	2.81
5% 3 cc	Contin.	2	1.98	2.54	2.57	2.78	2.79
10% 3 cc	Contin.	1	3.51	3.98	3.94	3.30	3.70
Meat:							
Raw:							
10 gm	10	1	1.76	0.76	0.69	0.19	0.10
30 gm	10	5	2.71	1.25	1.55	0.26	0.36
Dry:							
1 gm	10	2	1.50	1.07	1.05	1.11	0.82
2.5 gm	10	2	3.13	1.55	1.31	1.33	1.38
5 gm	10	1	4.00	3.00	3.56	2.10	3.16
White bread:							
Fresh:							
2.5 gm	10	1	1.80	0.82	0.82	0.41	0.69
5 gm	10	2	1.76	0.97	1.07	0.57	0.52
10 gm	10	2	3.01	1.50	1.85	0.69	0.76
Dry:							
2 gm	10	3	2.63	3.17	2.73	2.11	2.33

* Given in 1 minute. Collection time, 2 minutes.

† Contin. = Continuously.

maxillary glands had lower thresholds than the parotid glands for sucrose, meat and bread, but not for weak concentrations of hydrochloric acid and magnesium sulfate. Weak stimulation by sodium chloride affected the parotid and submaxillary glands to about the same degree. With strong stimuli, except acid, the submaxillary secretion exceeded that of the other glands in volume.

The glands have, however, different secretory capacities which, on the basis of their responses to pilocarpin, had approximately the following

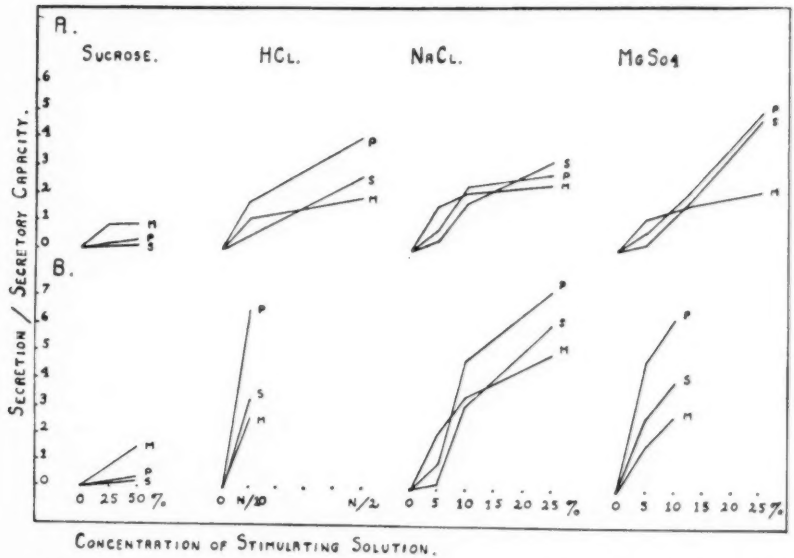

Fig. 1. The salivary and mucous glandular responses of dog 6 (F., wt. 10.3 kgm.) to taste stimuli, charted on the basis of volume of secretion/total secretory capacity of the gland. A, 1 cc. given in 1 dose. B, 1 cc. given continuously in 5 minutes. Collection time, 5 minutes. M, oral and pharyngeal mucous. P, left parotid glandular saliva. S, left submaxillary glandular saliva.

ratios: submaxillary gland : parotid gland : oral and pharyngeal mucous glands = 1.0:0.6:1.2. By dividing the quantities of secretion given in table 2 by these factors we obtained figures indicating the relative degrees of response of the glands, which have been plotted in figure 1.

The mucous glands respond more actively than the salivary glands to weak stimuli, with the exception of acid. The curves of mucous secretion in response to single doses of the stimulating solution level off very early, reaching about the same height for stimulation by HCl, NaCl, or $MgSO_4$. In this respect they differ from the curves of submaxillary and parotid glandular secretion which rise at a different rate for each of the taste

stimuli. The more uniform character of the curves of mucous secretion may have been due in part to the low threshold of the mucous glands for

TABLE 3

The conditioned reflex responses of the salivary and of the oral and pharyngeal mucous glands to meat and bread

Dog 6. Female. Wt. 10.3 kgm. Secretion from fistulae in grams

	ESOPHAGUS	LEFT SUBMAXILLARY	RIGHT SUBMAXILLARY	LEFT PAROTID	RIGHT PAROTID
Bread conditioned reflex					
Control 5 minutes	0.10	0.02	0.03	0	0
Control 5 minutes	0.11	0.02	0	0	0
Saw and smelled bread for 5 minutes	1.74	0.77	0.65	0.18	0.10
Given 10 grams bread in 10 pieces during 5 minutes	3.05	1.50	1.30	0.70	0.70
Succeeding 5-minute period	0.32	0.04	0.19	0.02	0.02

TABLE 4

The effect of water deprivation upon the reflex responses of the salivary and oral mucous glands to acid

Food and water daily except as indicated. Stimulus, 2 cc. N/10 HCl orally in 60 seconds. Collections made at the end of 2 minutes.

DAYS	REMARKS	MUCOUS	SECRETION FROM GLANDS			
			Submaxillary		Parotid	
			Right	Left	Right	Left
		grams	*grams*	*grams*	*grams*	*grams*
1	Control. Not thirsty (Av. 3 exp.)	2.51	2.65	2.80	2.40	2.32
2	Water withheld following this exp. (Av. 2 exp.)	2.67	1.78	2.22	2.19	2.36
3	Thirsty. Water withheld (Av. 3 exp.)	1.77	1.63	2.09	1.66	1.72
4	Thirsty. Water withheld (Av. 2 exp.)	2.07	1.90	1.92	1.78	1.98
5	Apparently very thirsty. Feeding and subcutaneous fluids after exp. (Av. 3 exp.)	2.19	1.23	1.41	1.70	1.73
6	Feeding and subcutaneous fluids. No experiment					
7	Not thirsty (Av. 2 exp.)	2.11	2.36	2.44	2.26	2.09
8	Not thirsty (Av. 2 exp.)	2.60	3.01	2.80	2.35	2.62

reflex mechanical stimulation, and be related to the licking and swallowing movements which accompanied the placing of any stimulating substance in the mouth. The parotid gland secreted at a higher rate in proportion

to its estimated secretory capacity than did the submaxillary gland in response to both taste and food stimuli, but especially to acid.

Conditioned reflex secretion of both saliva and mucus was easily established. In table 3 we give an example of a food-conditioned reflex, together with the corresponding unconditioned response. The conditioned reflex of the mucous and submaxillary glands was a replica in miniature of the unconditioned reflex upon which it is based (5e). The conditioned response of the parotid glands was very small when compared with the unconditioned reflex secretion.

TABLE 5

The secretory response of the salivary and mucous glands of the dog to pilocarpin during a period of water deprivation

DAYS	REMARKS	SECRETION OF GLANDS										
		Initial collection: 5 minutes					Total secretion: 1 hour					
		Mucous	Submaxillary		Parotid		Mucous	Submaxillary		Parotid		
			Right	Left	Right	Left		Right	Left	Right	Left	
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	
1	Control. Dog not thirsty	3.16	8.53	7.69	4.76	4.60	17.16	18.74	18.20	9.65	8.62	
2	Control. Dog not thirsty	4.75	7.85	7.04	4.00	3.84	20.36	14.31	14.36	9.43	7.98	
3	Control. Dog not thirsty	6.30	7.63	7.61	4.54	5.35	22.22	15.03	16.54	11.67	10.82	
4	No water or food given. Experiment at first appearance of thirst. Retching during first collection period	6.34	8.94	7.69	4.69	6.06	24.90	19.90	19.30	11.13	11.73	
5	No water or food given. Moderately thirsty	4.49	6.60	6.88	4.32	4.88	20.39	16.36	16.97	9.41	9.52	
6	Thirsty. Food, water, and subcutaneous fluids given after the experiment	3.81	4.26	4.14	2.32	1.92	20.07	15.38	16.43	6.77	5.50	
7	Given food and water. Moderately thirsty	7.64	7.10	6.20	4.10	4.15	24.55	21.66	21.77	10.77	9.43	

The effect of water deprivation upon the secretion of mucus. It is a well attested observation that the salivary secretion is decreased by dehydration. We compared the effects of water deprivation in dog 6 upon the oral and pharyngeal secretion of saliva and of mucus induced by the administration of 2 cc. N/10 HCl acid by mouth and by 0.2 mgm./kgm. of pilocarpin intravenously. After control determinations on successive days had established the normal level of response the dog was deprived of water for 3 days. Observations were continued during the periods of water dep-

rivation and recovery. With acid stimulation (table 4) there was a lower average secretion during water deprivation. However, even on the third day, when thirst appeared severe, certain individual collections were within the limits observed during the control studies. The secretion of the mucous glands was less affected than that of the salivary glands.

With stimulation by pilocarpin (table 5) retching occurred on the first day of water deprivation during the first five minutes of the experiment, a fact which may account for the high total secretion noted on this day. Throughout the period of deprivation the responses of the parotid glands were depressed, but those of the submaxillary and mucous glands, after the first five minutes of the collection period, were not. Consequently, the total secretion of submaxillary saliva and of mucus for one hour, although low, was within the limits of the controls, even on the third day of deprivation.

CONCLUSIONS

1. The oral and pharyngeal mucous glands resemble the salivary glands in their secretory responses to drugs.

2. Like the salivary glands they do not secrete continuously, but only in response to a large number of unconditioned and conditioned reflex stimuli.

3. The mucous glands are much more responsive to mechanical stimulation of the mouth than are the salivary glands.

4. They have lower thresholds than the salivary glands to weak food and taste stimuli, with the exception of acid. With stronger food and taste stimuli the salivary glands secrete more than the mucous glands.

5. During periods of water deprivation the mucous glands maintain their normal rate of secretion longer than do the salivary glands.

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THE EFFECT OF ACCELERATOR NERVE STIMULATION AND OF ADRENALIN ON RECOVERY FROM VENTRICULAR FIBRILLATION IN THE CAT

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Numerous attempts have been made to demonstrate an effect of the cardiac nerves upon ventricular fibrillation. Much of the earlier work was casual in nature and is somewhat suspect as the tendency for the fibrillating hearts of smaller mammals such as cats to recover spontaneously was neither adequately recognised nor thoroughly controlled (MacWilliam, 1887; Garrey, 1924). In animals with larger hearts such as dogs recovery is not anticipated and is considered unusual when it does occur. All aspects of cardiac fibrillation both auricular and ventricular are thoroughly reviewed by de Boer (1923), Garrey (1924) and Winterberg (1926) and this literature will not be referred to here. Workers are by no means in agreement as to the results of separately stimulating both the accelerators and vagi. The majority report greater ease in the establishment of fibrillation or a tendency to prolong it once it is established on stimulating the accelerator nerve, and the opposite effects on stimulating the vagus. In our experiments we find that stimulation of the accelerator nerve definitely hastens recovery from fibrillations produced by directly faradizing the cat heart.

PROCEDURE. Throughout our work cats under dial[1] anesthesia were used. Arrangements were made to record the blood pressure with a mercury manometer attached to a cannula in either the right or left carotid, the heart was exposed by opening the thoracic cavity, artificial respiration started and the pericardium removed. The right stellate ganglion was exposed and the accelerator nerve was prepared for stimulation. Fibrillation was produced by applying a faradic current to the base of either the right or left ventricle near the interventricular septum. The strength of current employed was varied with the ease with which the ventricles were thrown into fibrillation. Once the desired strength was found this was usually maintained constant throughout the experiment. Recovery from

[1] Furnished by courtesy of Dr. C. C. Haskell, Ciba Company, New York City.

fibrillation usually occurred in the cat ventricle in less than a minute after the initial faradization. Occasionally recovery was delayed. We have, for example, witnessed spontaneous recovery after fibrillation had persisted as long as nineteen minutes. However, only hearts exhibiting the shorter recovery time were used in these experiments.

Our method was as follows: the ventricles were thrown into fibrillation by a two second faradization, allowed to recover and the blood pressure permitted to return to its pre-fibrillation level. Once this level had been reached the right accelerators were tetanically stimulated and, after a slight rise in blood pressure had attested to their efficacy, the ventricles were again faradized, the accelerator stimulation being continued throughout the subsequent fibrillation and stopped only after the ventricles had recovered. After a short time the process was once more repeated, this time without stimulation of the accelerators. This routine was continued as long as the heart remained in good condition, every other fibrillation occurring during simultaneous stimulation of the accelerator nerve. In this way it was possible to obtain records of the duration of several successive fibrillations, thirty-two being the maximum number obtained in one animal. A continuous kymographic record of the blood pressure was taken from the start to the end of the experiment. At the beginning of the experiment the blood pressure was usually in the neighborhood of 100 mm. Hg and dropped slowly to a value of about 40 mm. Hg.

From this record the average length of the fibrillation during accelerator stimulation was determined and compared with the average length of the fibrillations when the accelerator was not stimulated. Any significant difference between the two was attributed to the effect of the accelerator nerve. The length of each individual fibrillation was calculated by measuring the time elapsing on the kymographic record between the initial fall in blood pressure (the beginning of faradization of the ventricles) and the first sign of its restoration as indicated by the sharp and sudden rise from the level prevailing during fibrillation.

In all but two of our animals atropine (0.2 cc. of a 1 per cent solution per kgm.) was administered before the experiment began. This was done to eliminate any chance of vagal stimulation, an involvement which sometimes occurs. In the two cases not injected with atropine (cats V and VI, table 1) it was very evident that the accelerator effects were not modified in any way by vagal influences. Consequently these animals serve as controls for those in which atropine was injected. As can be seen from inspection of table 1 there is no reason to believe that the injection of atropine in the doses employed has altered the effect of accelerator excitation.

Results. Invariably the effect of stimulating the accelerator nerve while the ventricles were fibrillating was to hasten the moment of recovery.

An example of this is given in figure 1. The data collected from six different cats, all females, are presented in table 1. As can be seen the average length of 61 fibrillations during excitation of the accelerator nerves was 10.8 seconds. When the nerve was not stimulated this figure, as determined from 60 fibrillations, rose to 26.3 seconds. Furthermore in each individual cat the average difference in fibrillation time due to accelerator stimulation is so marked as to leave no reasonable doubt as to the reality of the effect. As an example we might cite the data collected from cat IV, the lengths in seconds of the successive pairs of individual fibrillations

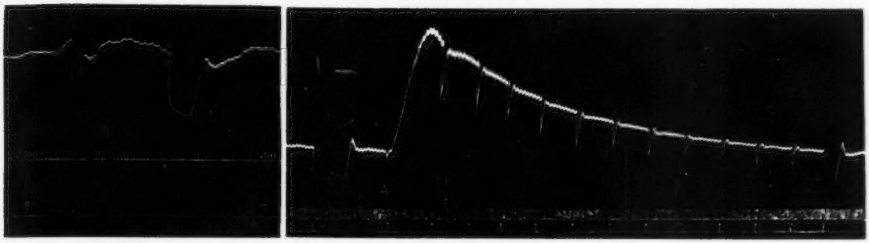

Fig. 1. Blood pressure tracing illustrating the effect of accelerator nerve stimulation on the speed of recovery from ventricular fibrillation. During fibrillation the blood pressure drops precipitously and recovers only with the recovery of the heart. Before and during the first fibrillation the accelerator nerves were stimulated (recovery time, 3 sec.); no stimulus to nerves was applied during the second fibrillation (recovery time, 12 sec.). Time signal indicates two second intervals. Lower signal marks times of stimulation: under first fibrillation; first rise, beginning of accelerator stimulation; first fall, beginning of faradization of ventricles; second fall, end of accelerator stimulation. Signals under second fibrillation mark beginning and end of faradization of ventricle.

Fig. 2. Blood pressure tracing illustrating the immediate recovery of the ventricles from fibrillation while under the influence of adrenalin. Adrenalin (0.3 cc. of 1:1000) injected at *A*. Faradization of the ventricles, marked by the signals, produced a fibrillation lasting 40 seconds before the adrenalin injection while afterwards, during the time the heart was under the influence of adrenalin, the fibrillations persisted only during the time of faradization (2 sec.).

in this animal being as follows: 28, 4; 26, 2; 62, 12; 12, 26; 48, 46; 24, 8; 15, 12; 18, 13; 22, 8 and 38, 12. The first figure in each pair is the length of fibrillation in seconds when the accelerator nerve is *not* stimulated and the second figure the length when the accelerator nerve is stimulated. The data are typical of all experiments except for the reversal in the fourth pair which is the only time this occurred in the six experiments.

In two cats, II and V, 5 per cent KCl was injected into the heart prior to the collection of the experimental data. In both of these animals after the initial fibrillations had persisted for several minutes, it was assumed that spontaneous recovery would not occur and KCl was injected to stop

the fibrillation before irreparable damage to the ventricular muscle occurred. In these two cats the ventricles subsequently showed prompt recoveries from fibrillation. The use of KCl in stopping ventricular fibrillation has been discussed at length by d'Halliun (1926), Hooker (1928) and Wiggers (1930). The administration of KCl in our experiments does not seem to have altered the shortening effect of accelerator stimulation upon the duration of subsequent ventricular fibrillation.

While in all six cats the fibrillation time was shortened by accelerator stimulation, the magnitude of the effect was clearly not constant (table 1). In cat II the stimulation decreased the average length of fibrillation time from 20.6 to 4.4 seconds, while in cat V it decreased it from 36.4 to 20.8

TABLE 1

CAT NUMBER	WEIGHT	NO ACCELERATOR STIMULATION				WITH ACCELERATOR STIMULATION			
		Number of fibrillations	Fibrillating time			Number of fibrillations	Fibrillating time		
			Average	Longest	Shortest		Average	Longest	Shortest
	kgm.		*seconds*	*seconds*	*seconds*		*seconds*	*seconds*	*seconds*
I*	Unk	17	10.6			14	5.6		
II*†	1.9	10	20.6	84	8	10	4.4	18	2
III*	2.0	12	19.6	50	16	12	9.8	20	4
IV*	2.4	10	29.3	62	12	10	14.3	46	2
V†	2.6	5	36.4	50	22	5	20.8	28	14
VI‡	3.2	6	41.3	56	18	10	9.6	18	4
Totals.....		60				61			
Averages...	2.4		26.3				10.8		

* Atropine injected.

† KCl injected into the heart.

‡ In cat VI four fibrillations are omitted from the averages obtained when the accelerator nerve was not stimulated. They were all of the prolonged type and would have unduly and unnecessarily increased the final result.

seconds. Cat II showed a greater difference because included in the averages for accelerator stimulation were a number of instantaneous recoveries, i.e., immediate recoveries on cessation of the faradization of the ventricles.

In a sense these experiments serve as their own controls, but to avoid the possibility of unsuspected factors influencing the results a different set of control data was collected. These consisted simply in the records of a series of fibrillations in six cats, *none* of which were influenced by accelerator nerve stimulation. The data were then treated *as if* the even numbered fibrillations were done during stimulation of the accelerator nerve, the average fibrillation time of the odd numbered fibrillations being calculated and compared with the average fibrillation time of the even numbered fibrillations. The results are presented in table 2. The differ-

ence between the two sets of averages obtained is hardly significant and is in striking contrast to that obtained from the experimental animals presented in table 1.

An inspection of table 1 and table 2 also discloses the probably significant fact that the average length of fibrillation is directly proportional to the weight of the cat, and therefore presumably to the size of the ventricles. Moreover the average fibrillation time in table 1 (26.3 sec.) is greater than that in table 2 (12.9 and 14.1 sec.). This is correlated with, and undoubtedly due to, the difference in average weight (2.4 kgm. as contrasted with 1.7 kgm.).

TABLE 2

CAT NO.	WEIGHT	ODD FIBRILLATIONS				EVEN FIBRILLATIONS			
		Number of fibrillations	Fibrillating time			Number of fibrillations	Fibrillating time		
			Average	Longest	Shortest		Average	Longest	Shortest
	kgm.		*seconds*	*seconds*	*seconds*		*seconds*	*seconds*	*seconds*
I	1.4	19	5.2	17	2	18	6.6	22	2
II	1.4	11	6.6	12	2	11	9.5	20	2
III	1.6	9	11.9	32	6	9	12.0	38	5
IV	1.7	11	9.2	18	5	12	11.2	19	7
V*	2.0	12	18.1	28	12	12	18.3	30	16
VI	2.3	14	26.3	88	16	14	27.1	88	10
Totals.....		76				75			
Averages...	1.7		12.9				14.1		

* KCl injected into the heart.

Since adrenalin is held to have the same effect upon tissues as stimulation of the sympathetic nerves supplying these tissues it seemed worth while to test the effect of adrenalin upon recovery from ventricular fibrillation. This was done by exposing the cat heart and selecting as before a strength of faradizing current which when applied to the ventricles established a persistent fibrillation. Adrenalin was now injected intravenously or directly into the heart and the ventricle faradized with a current of this identical strength. Typically the ventricles stayed in fibrillation only as long as the electrodes were applied. The few exceptions were seen in hearts which developed irregularities after the injection of adrenalin. Except for these aberrant cases lasting fibrillations could not be established until the effect of the adrenalin, as shown by a return of the blood pressure to its level before injection had completely worn off. The dose of adrenalin selected was usually 0.1 to 0.5 cc. of 1:1000. Large doses such as this were chosen not because they were necessary to produce the effect but because the effect of smaller doses was more transitory. We

have observed the effect with an intravenous injection of 1 cc. of 1:100,000 adrenalin. Figure 2 shows the typical result of many experiments.

From this it is clear that adrenalin prevents the establishment of ventricular fibrillation by faradization of the ventricles in the cat.

Discussion. On the whole the literature pertinent to this problem seems to favor the view that accelerator nerve stimulation and adrenalin predispose the heart to ventricular fibrillation as contrasted to our results which indicate that under similar conditions recovery occurs more promptly. In most of this work the heart was influenced by factors other than sympathetic effects. Thus Levy (1912), Brow, Long and Beattie (1930) and Nahum and Hoff (1934) observed a predisposition to fibrillation in animals under the depressing effects of chloroform or benzol while Schlapp (1933) obtained this result, following adrenalin injection, only during a severe alkalosis. These results may not be due to any specific effect of sympathetic stimulation or adrenalin but rather to some other factor, such as an increased load, affecting an already damaged heart muscle. Certain observations of ours are in accord with this view, specifically those few instances in which adrenalin injections were followed by obvious arrhythmias. Faradization of these hearts sometimes resulted, as has been previously mentioned, in the establishment of a persistent fibrillation rather than in a fibrillation followed by the usual instantaneous recovery.

Recently Hoff and Nahum (1935) reported the removal of the stellate ganglia and adrenal glands to decrease the susceptibility of the cat heart to ventricular fibrillation produced by sending a 110 volt alternating current through the body. Such hearts were restored to their usual susceptibility to fibrillation by the subcutaneous injection of adrenalin. The animals received neither chloroform or benzol. These results, while not strictly comparable with ours, because of the subcutaneous route of adrenalin administration and because of the method used in causing fibrillation, nevertheless appear to be in disagreement with those here reported. On the other hand auricular flutter and fibrillation has been shown by Rotherberger and Winterberg (1915) to be in no way increased by excitation of the accelerator nerves but on the contrary probably shortened.

At this time one can only speculate as to the mechanism by which sympathetic stimulation and adrenalin further the recovery of the cat ventricle from fibrillation. Possibly improved A-V conduction on stimulation of the accelerator nerves as noted by Bayliss and Starling, (1892); Gaskell (1886); Dale and Mines (1913) and Izquierdo (1929) may be involved. The demonstration by Woollard (1926) of sympathetic nerve endings in the ventricular muscle opens the way for a consideration of a direct effect on the muscle due to sympathin liberation from these endings.

To avoid any possible misunderstanding we feel it necessary to add that

the action of the accelerator nerves is not held in itself to be responsible for the recovery but is to be considered rather as a factor hastening this process.

The relationship between the size of the heart and persistence of fibrillation was first noted by MacWilliam (1887) when he observed the hearts of small animals to recover more readily from fibrillation than those of large animals. Porter (1898) showed that it was easier to stop an established fibrillation in pieces of heart than in the whole heart itself. Garrey (1914) extended these observations further and demonstrated that the length of cardiac fibrillation was proportional to the tissue mass involved. Thus it is not surprising to find, as we have done, that within the species itself the size of the heart determines the speed of recovery from fibrillation.

SUMMARY

1. In cats spontaneous recovery from ventricular fibrillation following faradization of the ventricles occurs in the large majority of animals.

2. Evidence is presented to show that the time of fibrillation varies directly with the weight of the cat (size of the heart).

3. Stimulation of the accelerator nerves during ventricular fibrillation in the cat shortens the duration of the fibrillation.

4. While the heart is under the influence of adrenalin, recovery from fibrillation following faradization of the ventricles is instantaneous.

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THE EFFECT OF COMPLETE RENAL INSUFFICIENCY ON THE ACTION OF PARATHYROID HORMONE IN THE DOG[1]

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Albright and associates (1929a, 1929b) and Ellsworth and co-workers (1932, 1934) have observed that the administration of parathyroid extract to human subjects immediately results in an increased urinary excretion of phosphates. These investigators attributed this to a lowering of the renal threshold for phosphates, and suggested that the fall in plasma phosphate, which follows the increase in urinary excretion, permits the subsequent rise in plasma calcium. Thomson and Pugsley (1932), however, have shown that the rise in serum calcium, which results from the intravenous injection of parathyroid hormone is not necessarily accompanied, or preceded, by a decrease in the total inorganic phosphate, or the PO_4 ions of the serum.

Selye (1932) has advanced the theory that parathyroid hormone directly stimulates the formation of osteoclasts in the bone, thus releasing calcium to the blood. This theory is supported by Pugsley and Selye (1933), who found in rats that the return of increased serum calcium to normal levels in the later stages of experimental hyperparathyroidism coincided exactly with the disappearance of osteoclasts from the bone marrow. Collip, Pugsley, Selye, and Thomson (1934) reported osteoclastic resorption of bone in bilaterally nephrectomized rats, which had been injected with parathyroid extract, but their observations were not supplemented by serum calcium determinations. They interpreted their findings as decisive evidence against the theory advanced by Albright and Ellsworth. The hypothesis which postulates the osteoclasts to be active agents of bone resorption has been criticized by Ham (1932a, 1932b) and by Schour and Ham (1934). Furthermore, Selye's theory is necessarily based upon the assumption that the blood is undersaturated with respect to calcium (Thomson and Collip, 1932) and is not in accord with evidence to the contrary advanced by Holt, La Mer and Chown (1925), and by Hastings, Murray, and Sendroy (1927).

[1] An abstract of this paper was presented before the American Society of Biological Chemists at Detroit, Michigan, April 10, 1935.

Goadby and Stacey (1934) have suggested that there are three possibilities as to the point of action of parathyroid hormone, namely, the kidney inorganic phosphate excretory mechanism, the kidney phosphatase, or the organic phosphate reserves in the blood or in the tissues. It would appear, also, from the foregoing observations that parathyroid hormone may mobilize calcium by acting directly on cellular elements in the bone.

The results of the experiments reported in this paper favor the view that the kidney plays a dominant rôle in the action of parathyroid hormone.

METHODS. Adult dogs, which had been on a standard diet[2] for several days, were used as the experimental animals. Fifteen hours before they were operated upon food was withdrawn. Ether anesthesia was employed and complete renal insufficiency was produced by extirpation of the kidneys, or by ligation of the renal arteries and veins. All of the animals were denied food during the period in which blood samples were taken, but were allowed water ad libitum several hours after the operation. Vomiting occurred in a few instances, but produced no noticeable effect on the blood constituents which were studied.

Two different parathyroid hormone preparations were used. One was prepared by the senior author's method (1930), and the other was the preparation known as parathyroid extract (Lilly). These extracts were administered in units as defined by Collip and Clark (1925). Serum inorganic phosphate was determined by the method of Fiske and Subbarow (1925) as modified by Hauch and Koch[3] for small quantities of blood. Serum calcium was determined by the Kramer and Tisdall method as modified by Tweedy and Koch (1929).

The animals were killed by intracardial injection of chloroform soon after the last blood sample was taken. Autopsies were performed and the tissues were placed in formaldehyde for fixation. Our usual procedure was to examine the tissues for necrosis and calcification, and not for lesser degenerative changes. For this purpose paraffin sections were made, and stained with hematoxylin and eosin.

DISCUSSION OF RESULTS. *Bilaterally nephrectomized controls.* Since 4 of the 5 control animals (dogs 2, 3, 4, and 5, table 1) showed a rise in serum calcium of 2.32, 2.21, 1.96, and 1.75 mgm. per cent, respectively, it was necessary to attempt to demonstrate an increase of 4 to 5 mgm. per cent in the serum calcium after removal of the kidneys, and the injection of parathyroid extract, in order to produce indicative evidence of hormone action.

Bilateral nephrectomy followed by injection of parathyroid extract. In order to obviate as much as possible the toxic effects which are known to develop steadily as a result of extirpation of the kidneys, an attempt was

[2] A commercial preparation known as Fox Chow.

[3] Personal communication to the senior author.

made to bring about a rapid mobilization of calcium into the blood (dog 6, table 2) by the intravenous injection of two hundred units of parathyroid

TABLE 1

Serum calcium and inorganic phosphorus after bilateral nephrectomy

TIME	DOG 1 WEIGHT, 11 KILOS		DOG 2 WEIGHT, 18 KILOS		DOG 3 WEIGHT, 15 KILOS		DOG 4 WEIGHT, 12 KILOS		DOG 5 WEIGHT, 13 KILOS	
	Serum Ca	Serum inorganic P	Serum Ca	Serum inorganic P	Serum Ca	Serum inorganic P	Serum Ca	Serum inorganic P	Serum Ca	Serum inorganic P
hours	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
−1	12.48		11.46	5.90	11.09	5.00	11.26	5.76	11.68	4.85
2	12.23	7.04	11.12	5.17	11.36	9.22	12.33	6.30	12.53	4.85
4	12.48	10.16	12.62	3.76	11.50	11.60	13.22	9.60		
6	12.48	12.00	12.72	9.22	11.60	11.82				
9	12.48		12.96	10.78	11.80	11.20	13.15	10.70		
24	10.82	14.62	13.78	16.66	13.30	11.20	13.11	15.00	13.43	8.84
			12.82	16.80						
			11.46	31.08						

TABLE 2

Serum calcium and inorganic phosphorus in bilaterally nephrectomized dogs after injection of parathyroid extract

TIME	DOG 6 WEIGHT, 10 KILOS			DOG 7 WEIGHT, 16 KILOS			DOG 8 WEIGHT, 14 KILOS			DOG 9 WEIGHT, 13 KILOS		
	Hormone, dose	Serum Ca	Serum inorganic P	Hormone, dose	Serum Ca	Serum inorganic P	Hormone,* dose	Serum Ca	Serum inorganic P	Hormone,* dose	Serum Ca	Serum inorganic P
hours	*units*	*mgm. per cent*	*mgm. per cent*	*units*	*mgm. per cent*	*mgm. per cent*	*units*	*mgm. per cent*	*mgm. per cent*	*units*	*mgm. per cent*	*mgm. per cent*
−1		12.45		150	10.92	5.94		10.63	4.82		11.11	3.00
¼	100 (i)†											
1										100	11.17	4.44
2		11.07	5.35		10.53	11.00	100	11.31	4.23			
4	100 (i)	11.95	6.00		11.94	6.06						
6		12.04	8.00	75	11.26	10.70						
8		12.14	9.58									
11	50	11.02										
16								13.82	8.40		13.03	9.60
24		9.56	15.58		12.18	12.62				100	12.26	9.23
48								12.87	7.97		13.87	11.50
72								11.28	9.40		13.14	12.56

* Parathyroid extract (Lilly).

† Administered subcutaneously unless designated (i) intravenously.

extract in divided doses. The serum calcium did not rise within the first eleven hours, and fell during the last thirteen hours, although an additional 50 units of parathyroid hormone were injected, subcutaneously. It will

be noted also that convincing evidence of hormone action was not obtained when huge doses of parathyroid extract were injected subcutaneously (dogs 7 and 9, table 2).

Ligation of the renal arteries and veins followed by injection of parathyroid extract. In three other animals (dogs 10, 11, 12) complete renal insufficiency was produced by ligation of the renal arteries and veins. Immediately afterward each animal was injected, subcutaneously, with 100 units of parathyroid extract. There was what might be regarded as a significant

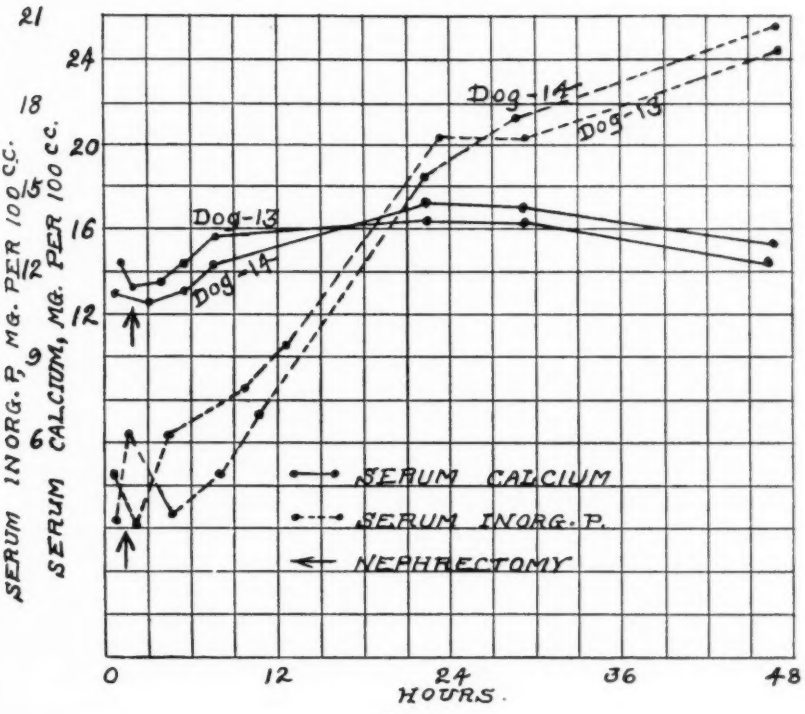

Fig. 1

rise in serum calcium in only one of these animals. In this animal (dog 12) a rise of 3.3 mgm. per cent in the serum calcium occurred within the first six hours. At that time a second dose of 100 units was injected, subcutaneously, but no further increase in the serum calcium occurred. Although it cannot be stated positively that the rise in serum calcium was due to the effects of nephrectomy alone, it does appear from the results obtained (tables 1 and 2) that the evidence indicating hormone action is slight and doubtful.

Hypercalcemia followed by bilateral nephrectomy. Collip and associates (1925) have shown that while the serum calcium in the normal dog is elevated as a result of the injection of parathyroid extract, the animal is more responsive to successive doses of the extract. They have shown also that parathyroid hormone overdosage in the dog leads to venous engorgement and hemorrhage in the stomach, while Hueper (1927) has described the production of lesions and calcification in the stomach and other organs under the same conditions. It appeared to us that these effects of excess parathyroid hormone action should be demonstrable in the nephrectomized dog, and that if such animals had an elevated serum calcium at the time of nephrectomy they should be still more susceptible to parathyroid hormone overdosage.

Hypercalcemia was induced in two dogs by the injection of parathyroid hormone 17 hours before bilateral nephrectomy, and the change in serum calcium and serum inorganic phosphate was followed during the succeeding 48 hours (dogs 13 and 14, fig. 1). Dog 13 also received 100 units of parathyroid hormone immediately after nephrectomy, but dog 14 received no additional hormone. It will be observed that the high concentration of serum calcium did not prevent a rapid increase in serum inorganic phosphate, which most probably occurred as a result of bilateral nephrectomy alone; and conversely, that the serum calcium level was well maintained in the presence of a rapidly increasing concentration of serum inorganic phosphate. It will also be observed that the magnitude and duration of the hypercalcemia were about the same in the two animals.

Collip (1925) and Thomson and Collip (1932) have stated that they are inclined to ascribe the chief rôle in the production of the pathological picture of parathyroid hormone overdosage in the dog to the simultaneous presence of hypercalcemia and hyperphosphatemia. We observed no gross signs of hormone overdosage, such as venous engorgement and hemorrhage in the stomach. Furthermore, we found no necrosis or calcification in either the heart or the stomach, although hypercalcemia and hyperphosphatemia had been present, simultaneously, for many hours. It would appear from our observations that the complete deletion of kidney function protected these animals from the overdosage effects of parathyroid hormone.

SUMMARY

1. The increase in serum inorganic phosphate, which follows complete deletion of kidney function, is unrestrained by a hypercalcemia previously induced by the injection of parathyroid hormone.

2. Complete deletion of kidney function protects the dog from the overdosage effects of parathyroid extract.

3. The mobilization of the calcium stores of the body into the blood by parathyroid hormone is dependent on kidney function.

The authors are indebted to Eli Lilly and Company for the parathyroid extract (Lilly) used in these experiments, and to Swift and Company for the collection of parathyroid glands used in the preparation of parathyroid extract. They are also indebted to Messrs. C. Vicens-Rios, G. H. Smullen, and L. Herdegen for their assistance in the operations and autopsies.

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ON THE RESPIRATORY CENTRE

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Lumsden (1923) suggested that the respiratory centre was divisible into three or four parts; only the most caudal of his centres, the gasping centre, lay below the striae acusticae. The work of numerous observers has shown that Lumsden's work is inaccurate,—Schoen (1929), Teregulow (1929), Henderson and Sweet (1929); and Henderson and Sweet particularly, have shown that respiration, normal in type, occurs in cats even if complete transverse sections are made below the striae acusticae. Such respirations are usually slower than normal, but adequate. It is of course clear that the medullary respiratory centre is, or may be, influenced in its activity by fibres reaching it from higher levels.

Since the work of Henderson and Sweet, the authors of this paper have been engaged intermittently in attempting to delimit the respiratory centre or centres and trace the afferent and efferent connections. All experiments have been made in cats and in all cases the animals have been young, incompletely developed animals and from 1.5 to 2 kgm. in weight. Operations have been carried out under urethane and ether. Before making incisions a clear view of the area of the medulla involved was obtained, either by the complete ablation of the cerebellum, using the method described by Henderson and Sweet, or more usually by removing the central portion of the occipital bone with a rongeur and the central portion of the cerebellum with a scoop. All bleeding was arrested. Sections were made with small splinters of safety-razor blades, using a gentle sawing motion. After the conclusion of the experiment the head of the animal was placed in formalin, and only after thorough fixation was the area involved removed. If the gross examination warranted it, the material was cut in serial sections, usually transverse, stained and mounted in series and examined by E. H. C.

In the serial sections, transverse lesions were often hard to delimit. The lesion rarely lay in the plane of the sections; if there was diffusion of blood this often occurred in another plane than that of the lesion and obscured the lesion proper. Further, such a fine incision, if not accompanied by bleeding, often produced such slight changes that only careful scrutiny could trace its course and extent. Indeed, in some cases this proved impossible.

A complete series of 30μ sections through a normal medulla was prepared and from this a plan of the various important structures was made. In making the plan, 10 per cent was allowed for shrinkage. Owing to the great kindness of Prof. J. W. Papez (1929) who published several sections through the medulla of the cat in his book, we have been provided with the serial numbers of the sections published, and also the thickness of the sections cut by him. Using these data, we have been enabled to check our plan against his figures. The plan thus prepared forms the basis for

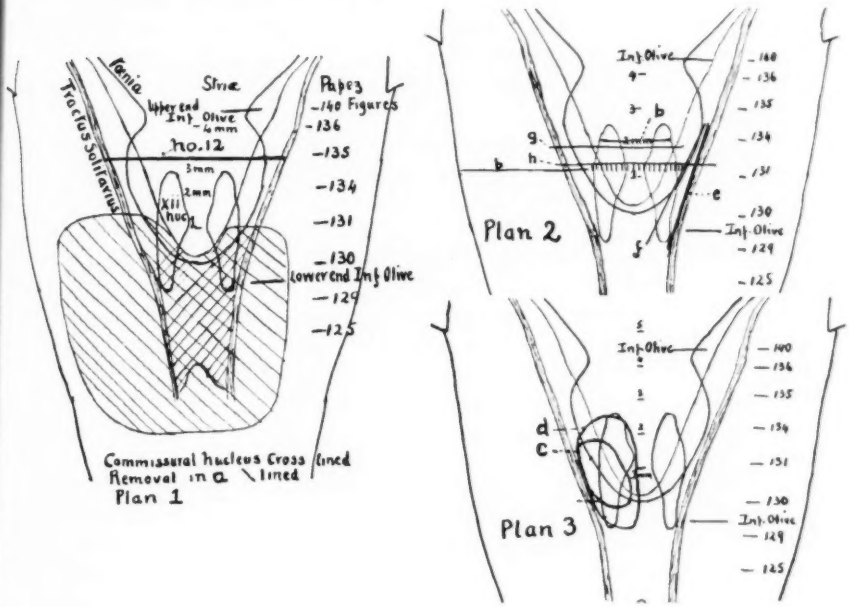

Fig. 1. Plan to scale of the medullary area prepared as described in the text, showing the relative positions of the important structures referred to in the text, and also the levels of the sections published by Papez.

figure 1. Further, as in the reproduction of photographs of the lesions in our preparations, much detail would not appear, it seemed advisable to plot these lesions on photographic reproductions of Papez' figures when possible, since this would enable any person interested to compare our figures directly with Papez' published ones, and hence obtain the relation of our lesions to other structures. This procedure also enabled us to present our results with the use of fewer figures.

Typical and selected experiments only have been described and assigned arbitrary letters for convenience of reference.

We would like to support the conclusions drawn by Porter, namely, that the cutting of nerve tracts does not necessarily produce an inhibition of the cells from which they arise or to which they run. Usually a lesion caused either no effect or a very temporary one, but in some cases respiration failed gradually during a few breaths. In these cases the failure was probably due to the effect of an attendant hemorrhage or interference with blood supply and was very obviously so produced in certain cases. In most such cases more or less prolonged artificial respiration leads to a recovery of function. If the lesion mechanically damages respiratory cells, they of course may not recover, and it is always difficult to estimate what tissues have thus been injured, but certainly cells which lie within 200 micra often function normally and cells lying even closer may retain their function.

The method has however certain disadvantages; that of identifying the lesions is one. The more important is that the lack of function may be due to the pressure produced by the diffusion of blood. The third lies in the great difficulty in exactly repeating any experiment. Nevertheless, this method has been almost exclusively employed, as it was found experimentally that electrical stimulation might give equivalent results from widely separated points, probably owing to stimulation of fibre tracts. The thermocautery has been used; again it appeared almost impossible to produce lesions comparable in position and depth, and ample evidence was acquired that temporary depression was due to the heat reaching cells beyond the area destroyed, and of course such damage may be invisible.

Henderson and Sweet found that respiration of a normal character persisted after a transverse section below the striae acusticae. Complete transverse lesions cut the descending vasomotor pathways and entailed a severe fall in blood pressure, but sections involving the medial third of the medulla, as was reported by Sweet and Henderson, do not have these defects. In their experiment, no. 12, plan 1, a dorso-ventral lesion which was limited laterally by the central portion of the nucleus ambiguus and the uncinate fasciculus and lay at a level a little caudal to Papez' section 135, i.e., cephalad to the hypoglossal nucleus and about at the junction of the upper and middle quarters of the inferior olive, left respiration intact and normal in character.

This work shows then that the respiratory centre lies below the striae acusticae and probably lower than the cephalic extremity of the hypoglossal nucleus.

Two possible sites have been suggested for the respiratory centres in this area. The first, that of von Bechterew (1908), who suggests that it lies in the reticular formation about the level of the hypoglossal nucleus; and the second that of Ramón y Cajal (1909), that it lies in the commis-

sural nucleus. The a priori reasons for the latter suggestion are obvious. Vagus afferents play an important part in respiratory control. These fibres enter the nucleus of the tractus solitarius and at the same or at a lower level synapse with its cells, from which fibres pass to the respiratory centre. Now the two nuclei of the tractus solitarius unite across the midline below the obex, forming the commissural nucleus, whose area is shown in plan 1, figure 1. From the lower parts of this nucleus, the solitary tracts again diverge and could form the paths of efferent respiratory impulses. The validity of this hypothesis was tested by clipping away with scissors or cutting away with the knife, the whole of this nucleus. Cat *a* was the most successful of these experiments, as no portion of the commissural nucleus was found histologically. The removal was completed at 11:10 a.m. and respirations became small and inadequate. Artificial respiration was given till 1:29, when the respirations were smaller than normal at about twice the normal rate and showing slight irregularities of a gasp-like character. Respiration, however, improved and at 2 p.m. it was more regular but still fast. Blood pressure had been low throughout this period and was at this time only 35 mm. Hg. An intravenous injection of 0.1 mgm. adrenaline caused some reflex slowing and decrease in depth, and respiration was somewhat more irregular. In other cases only an isolated group of some 100 cells was left. All these cases showed more or less lasting respiratory depression as a result of the operation. In cat *a* the area cut away is shown in plan 1, and the depth of the lesion is indicated at two levels in sections 129 and 130 in figure 2. As will be seen in section 130, the lesion reaches nearly to the level of the hypoglossal nucleus and did so over practically the lower half of this nucleus. There was a prolonged depression of respiration and no doubt some damage was done to some respiratory cells. These experiments, then, lend support to the suggestion of von Bechterew that the respiratory centre lies in the reticular substance, ventral to the xii nucleus, and demonstrate completely that the respiratory cells do not lie in the commissural nucleus.

As a means of delimiting the respiratory area, we resorted to longitudinal and transverse sections. We felt ourselves justified in considering that the respiratory centres lie in the medial portion of the medulla, as longitudinal sections lateral to this region did not abolish respiration. For example, in cat *e*, a longitudinal cut was made, whose position is shown in plan 2 and sections 131, 130 and 129. Respirations became somewhat apneustic in type for a few minutes and then slower than normal and ample, apparently normal in type as both sides of chest and diaphragm participated in the movements. The cut at the level of Papez 131 lies just medial to the solitary tract at its dorsal end and is also medial to it in 130.

In experiment *f* the cord was split in the midline over segments 3–6,

(At postmortem it was found that in segments 3–4 the lesion was very slightly to the left of the midline; in the others, however, in the midplane.) A longitudinal incision on the right side was then made from a point about 2 mm. cephalad to the obex and lateral to the xii nucleus. The lesion extended ventrally to the inferior olivary sac (plan 2, sections 131 and 130). Caudad the lesion did not penetrate so deeply, being only to the level of the xii nucleus and approaching the midline, so as to lie just medial to this nucleus. Almost the whole of this section was made without any

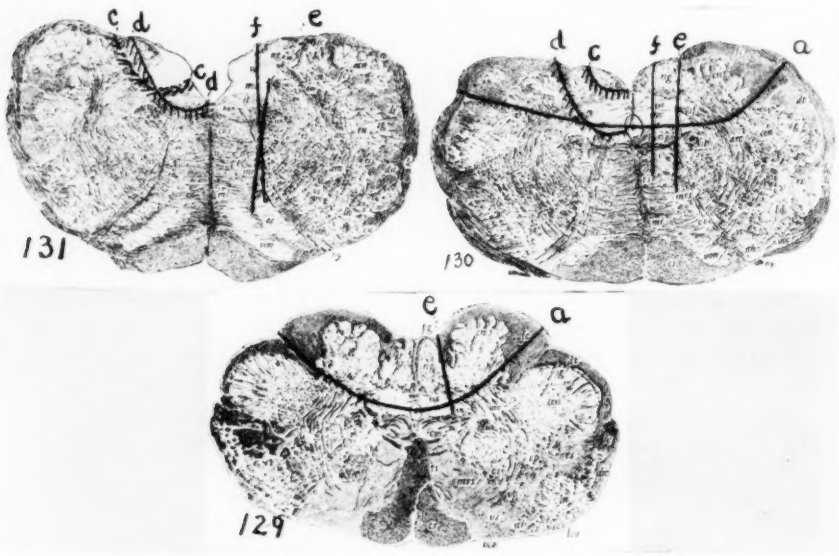

Fig. 2. Sections 131, 130, 129 are reproductions of the figures with corresponding numbers in Papez' book. The position of the lesions at the respective levels is shown. All the structures dorsal to the curved lines for cats *d*, *c* and *a* were either removed or destroyed. The striae on lines for cats *d* and *c* indicate the areas showing histological change due to heat.

apparent change in respiration, save a slight decrease in depth (respiration was recorded quantitatively) but a slight extension cephalad and the insertion of a small probe led to a definite decrease in depth and rate. Consequently artificial ventilation was employed for 20 minutes, respirations being then slow and weak. On opening the abdomen only the left diaphragm appeared active. The diaphragm was then split in the midline and the left half still moved and the right did not. The production of this lesion had apparently damaged a part of the respiratory mechanism.

From these and similar experiments the conclusion has been drawn that the respiratory cells lie in the medial third of the medulla.

In view of the conviction that the respiratory cells lay in the medial third of the medulla, transverse sections were made, involving the medial third only, with the purpose of defining the cephalad extent of the area containing the respiratory cells. The caudal limit must be set by the pyramidal crossing which begins only about one millimetre below the obex in cats of the size used.

In cat *g*, the lesion (plan 2) extended on the left through the fasciculus solitarius and on the right to just beyond the lateral margin of the xii nucleus. The width and level are shown in plan 2. Ventrally it extended on the left nearly to the inferior olive but on the right not so deeply. The transverse cut seemed to be about 2.3 mm. cephalad to the caudal end of the inferior olive or about 1.8 mm. cephalad to the obex at the junction of the upper and middle thirds of the xii nucleus. It lies between Papez' figures 131 and 134. In spite of a good deal of bleeding and definite diffusion of blood as seen in the sections, which led to inadequate respiration and the need of artificial ventilation for a time, the respirations became normal in rate and depth (recorded quantitatively). On being bled, gasps appeared, intermingled with normal respirations. Neck and shoulder muscles were involved.

In cat *h*, a transverse incision (level and width shown in plan 2) was made reaching lateral to the fasciculus solitarius on the left, and somewhat further on the right, and ventrally to the upper surface of the inferior olive on the left but not quite so deep on the right and lying about 1.4 mm. cephalad to the obex or about the junction of the upper and middle thirds of the xii nucleus. There was a good deal of suffusion of blood below and above the level of the section, and owing to a defect in the normal closure of the spinal canal which persisted as a split, the exact level was hard to identify, and hence the hatching below the line in plan 2 is intended to represent the area in the centre of the plan in which the lesion proper occurred. Respiration continued after the cut was made for a few breaths, which were about half normal depth and at half the rate and then failed. After 10 minutes artificial ventilation the cat breathed for two minutes, but inadequately. After a further hour's ventilation, the blood pressure was good and respiration was more regular and slow, but inadequate in depth. Another two hours' ventilation did not lead to any improvement; blood pressure was low. When gasps supervened, there were no movements of the neck or shoulder muscles. It seems very probable that this lesion had cut into the respiratory area, or that blood defusion had damaged certain of the respiratory cells. The failure of gasping movements of the neck and shoulder muscles, which has also been observed in other cases with low sections, even when gasp-like movements were made by the abdominal muscles, suggests that the innervation of certain of the accessory muscles arises from a higher level than that of the abdominal.

It would seem then that the respiratory cells are included in the medial

third of the medulla posterior to a level some 2 mm. cephalad to the obex in cats of this size, i.e., from a level more cephalad than the junction of the upper and middle thirds of the xii nucleus and extending to or below the obex.

Further evidence that the respiratory cells lie towards the midline may be drawn from a long series of experiments in which longitudinal incisions were made in the midline. Langendorff (1881) has frequently been quoted as saying that a midline incision through the medulla did not interfere with normal respiration, save that under other experimental procedures some evidence of independent action of the muscles of the two sides might occur. However, on looking up his work it is evident that his incisions were made from the obex cephalad. We have had the same experience, for instance, in cat *i* where an incision cephalad was followed by normal, though perhaps not so deep respiration, while when the cut was prolonged caudad for 1 mm., respiration ceased and even prolonged ventilation did not lead to its recovery. In other experiments incisions below the obex did not lead to failure, while when prolonged above it, failure was prompt, complete and permanent. At least a dozen experiments of this type were carried out.

There are two possible explanations for these observations: *a*, that the respiratory cells lie so close to the medial plane that the section produced sufficient mechanical damage as to cause arrest of their function; or *b*, that efferent fibres from the respiratory cells cross like those from the red nucleus before descending the cord. This latter suggestion is contrary to the evidence presented by Gad and Marinescu (1893) who burnt away areas of the medulla by means of small hot drops of glass. Unfortunately, they give no description of the extent or exact position of these lesions. Respiration ceased on the side of the lesion. This is of course possible if the lesion cut the respiratory path after crossing. We attempted to imitate these experiments by using a fine thermocautery. In two cases a distinct unilateral failure of respiration occurred, but on the opposite and not on the same side. The areas involved by these lesions are shown in plan 3 and in sections 131 and 130, lesions *c* and *d*.

Many of the experiments were indefinite. Further, there is evidence in the experiments of Porter (1894), Rothmann (1902) and other workers that a partial crossing, at all events, occurs at the level of the anterior horn cells. A section in the cervical cord may lead to the diaphragm moving normally or to failure on the same side. If, however, the opposite phrenic is cut, movement will begin in the apparently paralysed side. It may fairly be concluded from the experiments of these workers that the crossing at the anterior horn cell level in the cord is not dominant under normal conditions but in cases of a unilateral lesion may be adequate to produce an approximately normal diaphragmatic movement. Conse-

quently, clear cut results could only be obtained with certainty if the spinal cord were split in the midline so as to prevent crossing at the anterior horn level. Yet when this procedure has not been carried out, a failure only on one side requires consideration, as the presence of an anesthetic and of the operative procedures may lead to the respiratory impulses not crossing at the anterior horn cell level so as to produce respiration on the opposite side. This frequently occurred in the experiments carried out by the authors cited, and in our own cases mentioned below with cord lesions. While splitting the cord alone has no apparent effect on respiration, the operative interference does not leave the animal in as good a state as if it had not been performed. These experiments have not led to a positive conclusion as to whether a medullary crossing occurs or not. Further, it is always possible that a lesion such as that in cat *f*, quoted above, might have affected the fibres after they had crossed.

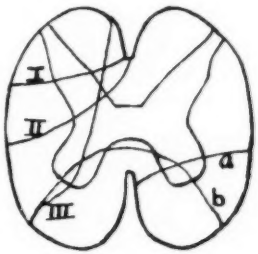

Fig. 3 represents a diagrammatic section of the cord at the 2nd–3rd cervical level. All tissue from the lines *I*, *II*, *III*, *a* and *b*, to the adjacent lateral or ventral surfaces was cut through.

As the course of the descending efferent fibres from the respiratory centre to the anterior horn cells is not generally recognized in textbooks of physiology, we repeated some of the experiments done by Gad and Marinescu, Porter, and Rothmann, with a somewhat varying technique. The recorded experiments of Rothmann and Porter were made on dogs and rabbits. Gad does not state the animal used. According to these authors, the descending efferent fibres lie adjacent to the anterior horn. Our experiments were of the following type. After baring the lower medulla and upper segments of the cord dorsally, a small section of the bodies of the 2nd and 3rd cervical vertebrae was removed and a section of the ventral white matter was made; this indeed often invaded the grey matter as in figure 3. No change in respiration was seen. A longitudinal section was then made in the cord so as to cut the pyramidal crossing; in only one case was this quite complete, but in others only a few of the lower fibres were uncut. This, too, did not affect respiration. Sections were made then in the dorsal and lateral area of one side at slightly different levels in the second and third segments of the cord. Section of areas such as that between I and the surface (fig. 3) or II or *b* and the surface have no effect, but a section between III and the surface (all these were made in the same animal and though at slightly different levels have been indicated on the same diagram) or between *a* and the ventral surface cause respiration to cease on the side of the lesion. The other side moves

amply and often, and adequate ventilation is obtained. If such an animal were bled somewhat, as in cat *j*, gasps appeared and these too appeared to be unilateral only. On section of the diaphragm in the midline only one side moved, either in respiration or in gasps. In cat *k*, 0.5 mgm. of nicotine had been given intravenously shortly before the section of the cord in the ventral area. Ipsilateral respiration ceased, on bleeding gasping occurred, the gasps involving only the heterolateral muscles of shoulder and neck as well as the diaphragm, in spite of the fact that twitches were occurring irregularly in the neck muscles of both sides, due to the nicotine. On splitting the diaphragm, only the heterolateral half moved, even in the gasps. While we do not deny that in the cat some crossing of descending fibres from the respiratory centre may occur, as Porter has so clearly shown in the dog, under the conditions of our experiments possibly due to the urethane used, they were not evident and the descending respiratory impulses appeared only to innervate anterior horn cells of the same side. Under the conditions of our experiments we saw no evidence of independent coördinated spinal respiration, even when the conditions were most favourable.

We also attempted to delimit the course of the fibres from nucleus of the tractus solitarius to the respiratory cells. These might leave the nucleus at the level at which the vagus afferents entered the nucleus and pass medially and caudally towards the respiratory centres. However, stimulation of the vagus after making one of the incisions across the medial third of the medulla, showed that either vagus produced the same effect as before the incision was made. Hence it is probable that either the synapse with vagus afferents occurs at the level of vagus entrance, the fibres to the respiratory centres descending in the tract, or the vagus afferents descended before synapsing. In either case the fibres from the tractus solitarius would pass almost medially from the tract to the respiratory cells. The following experiment selected from others, suggests that this is the course of the fibres. In cat *b*, a transverse lesion was made, cutting into both xii nuclei at a level corresponding Papez 134, about 2 mm. above the obex (plan 2) reaching deep down into the tectospinal tracts and becoming narrower than at the surface. In other experiments, wider sections were made, extending almost to the tractus solitarius at this level. After such sections, stimulation of both vagi were as effective as before the section. Yet such sections would fall below the level of the entrance of most vagus afferents into the medulla, consequently the fibres from nucleus solitarius do not pass medially and caudad to the respiratory cells. Then in this cat a second section was made at a slightly lower level, as shown in plan 2. This section lay about 1.25 mm. above the obex and extended from the surface dorsally to the left margin of the xii nucleus to the ventral margin of the 5th spinal root laterally and ventrally. It

thus cut the left tractus solitarius. This section almost completely abolished any effect of left vagus stimulation. Stimuli of the same strength had no effect, but stronger ones after a longer delay than normal had a slight inhibitory action. This section probably fell below the level of any vagus afferents and consequently either the vagus fibres or the descending fibres from the nucleus solitarius must descend in the tract before leaving it to pass almost medially to the respiratory centre.

SUMMARY

1. The respiratory area appears to lie in the medial third of the medulla from about the level of the junction of middle and upper thirds of the hypoglossal nucleus and is presumably limited below by the pyramidal crossing.

2. Its afferent fibres from the tractus solitarius pass medially from the tract to the respiratory cells.

3. The path descending to the anterior horn cells lies lateral to the anterior horn in the cord.

4. Certain experiments suggest that there may be a crossing of the descending fibres in the medulla at the level of their origin.

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GAS EQUILIBRIA IN THE LUNGS AT HIGH ALTITUDES[1]

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Most physiologists think that the passage of oxygen from the alveoli to blood depends on diffusion alone, but this opinion is not held universally. Haldane and Priestley (1) in their most recent contribution to this subject agree that under normal circumstances secretion may not occur, but believe that it is necessary in order to account for the survival of man at extreme altitudes. The basis for this belief consists of observations of the Pike's Peak party (2), in which Haldane had a part, of various laboratory experiments, and of experiences of mountain climbing expeditions.

Shortly before the Pike's Peak party, Krogh and Krogh (3) had improved the aerotonometric method of Bohr and reached the conclusion that oxygen enters the blood by diffusion. Further support was given the diffusion theory by the Cerro de Pasco party (4). The oxygen dissociation curve was found to shift to the left at high altitudes (contrary to the results of the Pike's Peak study) and on this account a comfortable gradient was furnished for the diffusion of oxygen. The most damaging blow given the secretion theory consists of the observation of the Barcroft party that natives of high altitudes may have an arterial saturation as low as or even lower than sojourners. This has been confirmed by Monge, Encinas, Heraud and Hurtado (5) at 3.72 km. and by Hurtado (6) at 4.54 km. These findings are not mentioned in the recent edition of "Respiration."

The early work on diffusion indicated that there is a pressure head of several millimeters across the alveolar epithelium. This was in harmony with the diffusion measurements of M. Krogh (7). Various reasons have been given by Böje (8) for believing that M. Krogh's method of calculating d_{O_2} from measurements of d_{CO} gives results which are too low. In particular, the studies of Hartridge and Roughton (9) suggest that d_{O_2} is at least one-half greater than the value deduced from d_{CO} measurements.

It has been supposed that there is a significant drop in pressure across the alveolar epithelium. In fact, by tonometric measurements, the partial pressure of oxygen in arterial blood in the resting man at sea level is

[1] For details regarding the financial support of these studies, see Keys, Hall and Barron, This Journal, **115**: 292, 1936.

about 20 mm. less than that of alveolar air. For several reasons, however, it is generally agreed that this is not a diffusion gradient. Haldane and Priestley (1) pertinently remark that if there is really such a gradient in rest, the diffusion theory must fall, for it would be impossible to account for the ten-fold increase in oxygen transfer in exercise. It is also significant that slight increases in the oxygen percentage in inspired air have practically no effect on the working capacity; one has to double the oxygen pressure before the effect becomes notable and then it can be explained by increase in dissolved oxygen in blood leaving the lungs.

The explanation advanced by Haldane and Priestley for these facts is that there is imperfect distribution of the air in the lungs in rest. If so, some blood may pass through incompletely oxygenated. The nature of the oxygen dissociation curve is such that a small amount of poorly oxygenated blood, when mixed with a large amount of fully oxygenated blood, will lower its percentage saturation but slightly, and its oxygen pressure considerably. The situation is quite different, however, when the arterial

TABLE 1

Illustration of the effects of mixing on the pO_2 of blood

	VOLUME	SEA LEVEL		5 KM.	
		Saturation	pO_2	Saturation	pO_2
	cc.	*per cent*	*mm. Hg*	*per cent*	*mm. Hg*
Arterial blood	90	98	120	75	40
Venous blood	10	68	35	45	24
Mixed blood	100	95	80	72	37

portion is only partially oxygenated, as will be clear from the hypothetical case given in table 1.

Various objections may be raised to the idea that in quiet breathing there is deficient oxygenation of some of the blood passing through the alveolar capillaries. If the argument is sound, it would appear that a moderate increase in the oxygen content of inspired air, with no change in breathing, would increase the arterial saturation. It should also be increased by overventilation at rest. Yet, Bock, Dill, Edwards, Henderson and Talbott (10) found that these measures leave the arterial oxygen saturation unchanged. As an alternative hypothesis they suggest that as the oxygenation of hemoglobin approaches completion processes within the capillary may be slow enough to prevent the attainment of equilibrium. The time required to reach equilibrium may become considerably less as the oxygen pressure is lowered. Roughton, in a personal communication, states that this hypothesis is compatible with his findings. He hopes to test it with the thermal method he and Bateman have developed (11).

Other possibilities are worthy of bare mention: *a*. There may be appreciable oxygen consumption in the lungs, an old suggestion which has been revived recently but which does not appear to merit serious consideration. *b*. Anastomoses may permit the by-passing of an appreciable amount of venous blood in the lungs. *c*. Some venous blood is returned to the left ventricle through the foramina Thebesii. *d*. The pH within the red cell as it leaves the alveolar capillary may be more acid than that reached in slow equilibration to the same saturation in vitro.

Our own studies in this field (12) led to the conclusion that the oxygen dissociation curve remains virtually unchanged at 3.05 and 4.39 km. While in agreement with the Pike's Peak party in this respect, we found no evidence for secretion by direct examination of arterial blood. A small but positive pressure head of oxygen was found just as in completely unacclimatized subjects breathing low oxygen mixtures at sea level. However, Haldane's belief in secretion was not fully invalidated because the altitude reached was quite moderate from a mountaineer's point of view.

The recent expedition to the Chilean Andes (13) has furnished an unusual opportunity to study this question. Our party became well enough acclimatized at 5.34 km. to carry on laboratory work without much handicap, and stays of from 2 to 6 days were made at 6.14 km. There are about 120 residents at 5.34 km., of whom about 80 climb each day to their place of work at 5.74 km. Some of the men who had spent years at this job were available as subjects.

The methods used for deriving the necessary data for construction of the physiological carbon dioxide and oxygen dissociation curves have been described by Keys, Hall and Barron (14). In addition to these data it is necessary to determine the CO_2 content, the O_2 saturation of arterial blood, and the composition of alveolar air at the time blood is drawn. The CO_2 content is applied to the CO_2 dissociation curve of arterial blood to obtain its pCO_2. The percentage saturation with oxygen is applied to the oxygen dissociation curve to obtain the arterial pO_2. The partial pressures in alveolar air are calculated as follows:

$$\left.\begin{matrix} pO_2 \\ pCO_2 \end{matrix}\right\} = \left.\begin{matrix} \text{per cent } O_2 \\ \text{per cent } CO_2 \end{matrix}\right\} \left(\frac{B - 47}{100}\right)$$

An unfortunate feature of this procedure is that the composition of arterial blood may be modified by abnormal breathing during the puncture. An index to the degree of disturbance can be obtained by collecting one sample of alveolar air before and another during the puncture. This test showed that some of our party, unfamiliar with the procedure, were upset by the first experiences. However, by the time the higher stations were reached, arterial punctures became commonplace and there was usually little difference in the composition of the two alveolar air samples.

A second aspect of the question worth consideration is that both alveolar air and arterial blood undergo rhythmic variation in composition, associated with breathing. It is well-known that samples of alveolar air vary in composition depending on the phase of respiration at which the forced expiration is made (15). We have followed the practice of collecting the sample at the end of normal expiration, for that is most nearly in equilibrium with arterial blood in so far as CO_2 is concerned (16). The magnitude of the rhythmic variation in composition of arterial blood has been revealed by the experiments of Kramer (17). With a dog under pernocton anesthesia the variation may range from 75 to 92 per cent saturation during the respiratory cycle. This rhythmic variation is entirely masked in our experiments because the withdrawal of 50 cc. of blood extends over several complete cycles.

In presenting the results in table 2 the observations at the two lower stations have been omitted. The results there were less satisfactory, partly because the subjects were less stable and partly because the technique, depending as it does on the efficient coöperation of several people, was not fully perfected. By the time Montt was reached (4.70 km.) consistent results were nearly always obtained. We had become able to predict very closely the arterial tensions from the composition of alveolar air and to prepare gas mixtures in the tonometer such that the correction of the CO_2 and O_2 curves was small in magnitude and high in precision.

The detailed findings show a small and usually positive ΔpCO_2. The mean values vary from +1.3 to +2.8 mm.; we are not inclined to attribute any significance to the fact that the largest differences were observed at the highest station. Since the question of CO_2 secretion rarely is raised, the most useful purpose served by these data is to demonstrate that the experimental technique was not much inferior to that attainable in a sea level laboratory under the most favorable conditions.

The observed values for ΔpO_2 were not always positive, notably at 5.34 km. where 7/10ths were negative, the mean value at this station being −0.8 mm. This must be looked upon as slender evidence for secretion since the collection of alveolar air at the end of normal expiration gives nearly the lowest pO_2 reached in the respiratory cycle. Furthermore, each of the two residents at this station from whom alveolar air samples were obtained had a positive ΔpO_2.

The individual variation in ΔpO_2 at the various stations will be discussed elsewhere in connection with the question of individual adaptation. It is enough to point out here that there is no simple relation between ΔpO_2 and arterial saturation. Other factors such as the position of the oxygen dissociation curve, the absolute value of the alveolar oxygen pressure as determined by respiratory control and a dependent function, the reaction of arterial blood, all play a rôle in determining the percentage saturation of arterial blood. A single illustration will make clear how these func-

TABLE 2

Relation between arterial and alveolar pCO_2 and pO_2 in rest

SUBJECT	DAYS AFTER ARRIVAL	ART. HbO_2	ART. CO_2	ART. pH_a	pCO_2, MM. HG			pO_2, MM. HG		
					Art.	Alv.	Δ	Art.	Alv.	Δ
A. Montt: Barometer = 429 *mm. Hg*; altitude = 4.70 *km.*										
		per cent	*m.-eq.*							
D.	0	73.7	19.0	7.47*	32.6	30.4	+2.2	42.7	45.0	+2.3
B.	1	70.8	18.2	7.45	30.8	32.8	−2.0	36.4	41.3	+4.9
T.	1	71.9	18.2	7.43	31.3	30.6	+0.7	40.7	39.8	−0.9
C.	4	82.8	16.4	7.51	23.5	25.3	−1.8	45.4	52.2	+6.8
M.	4	79.7	16.7	7.47	26.3	26.8	−0.5	47.0	49.8	+2.8
F.	5	83.0	15.8	7.50	24.0	24.9	−0.9	44.5	52.7	+8.2
E.	6	77.7	16.8	7.42	30.3	29.2	+1.1	40.5	44.2	+3.7
T.	7	75.0	18.4	7.39	34.3	30.0	+4.3	43.8	42.5	−1.3
K.	7	85.0	15.9	7.40	30.3	26.4	+3.9	47.2	47.8	+0.6
B.	8	80.7	17.6	7.40	32.9	28.3	+4.6	47.5	49.4	+1.9
D.	9	71.6	18.5	7.45	30.9	29.2	+1.7	40.4	43.6	+3.2
McF.	9	79.4	15.6	7.47	26.1	25.6	+0.5	43.2	51.0	+7.8
H.	11	83.2	15.7	7.52*	28.0	24.7	+3.3	46.8	50.6	+3.8
Mean		78.0	17.1	7.45	29.3	28.0	+1.3	43.5	46.9	+3.4
B. Visiting party at 'Quilcha: Barometer = 401 *mm. Hg*; altitude = 5.34 *km.*										
K.	3	78.7	13.8	7.46	22.9	22.9	0.0	43.7	44.7	+1.0
M.	3	79.1	15.8	7.41	29.3	25.9	+3.4	43.7	41.6	−2.1
B.	5	77.4	16.3	7.49	26.4	27.2	−0.8	43.0	41.5	−1.5
T.	3	72.8	17.4	7.39	34.0	29.0	+5.0	43.7	37.8	−5.9
C.	4	76.3	16.0	7.45	26.3	23.8	+2.5	41.7	45.7	+4.0
McF.	3	81.6	15.3	7.43	27.7	23.9	+3.8	50.4	47.5	−2.9
F.	5	79.2	14.9	7.42	27.0	25.7	+1.3	44.1	42.6	−1.5
H.	1	75.6	14.2	7.43	25.3	24.2	+1.1	43.4	43.1	−0.3
D.	4	65.4	17.9	7.40	31.8	29.0	+2.8	37.6	37.4	−0.2
E.	16	76.3	14.4	7.42	26.7	25.0	+1.7	39.9	41.3	+1.4
Mean		76.2	15.6	7.43	27.7	25.6	+2.1	43.1	42.3	−0.8
C. Residents of 'Quilcha										
C.		74.8	13.5	7.34	30.9			42.3		
M.		75.2	16.0	7.42	27.9			39.9		
An.		83.0	14.3	7.45	26.0			47.8		
A.		78.3	14.1	7.35	31.1			47.3		
F.		84.6	11.8	7.38	22.8	19.0	+3.8	52.0	54.1	+2.1
B.		70.2	15.1	7.36	31.7	27.5	+4.2	34.4	39.7	+5.3
H.		67.6	14.7	7.32	34.7			38.9		
Mean		76.2	14.2	7.37	29.3			43.2		

TABLE 2—*Concluded*

SUBJECT	DAYS AFTER ARRIVAL	ART. HbO_2	ART. CO_2	ART. pH_a	pCO_2, MM. HG			pO_2, MM. HG		
					Art.	Alv.	Δ	Art.	Alv.	Δ
D. Punta: Barometer = 356 *mm. Hg*; altitude = 6.14 *km.*										
M.	3	68.5	14.3	7.41	26.3	21.5	+4.8	37.9	40.5	+2.6
K.	6	73.0	12.5	7.41	23.0	20.0	+3.0	40.1	37.7	−2.4
M.	6	68.8	14.6	7.41	26.6	22.1	+4.5	38.9	39.0	+0.1
F.	1	64.2	14.1	7.47	23.1	23.0	+0.1	26.9	34.8	+7.9
T.	1	55.5	14.9	7.37	30.2	24.2	+6.0	32.1	32.6	+0.5
F.	2	67.7	12.2	7.46	20.3†			35.5†		
McF.	2	71.6	11.9	7.48	19.4	17.2	+2.2	39.4	42.6	+3.2
B.	0	62.2	14.3	7.43	26.3	23.0	+3.3	36.9	37.0	+0.1
H.	0	58.7	12.8	7.56	18.5	20.4	−1.9	27.9	37.2	+9.3
Mean		65.6	13.5	7.44	24.2	21.4	+2.8	35.0	37.7	+2.7

* Experimental value with the glass electrode. All other pH_a values have been calculated by the Henderson-Hasselbalch equation.

† Not included in the average.

tions can account for a high oxygen saturation without the help of a favorable diffusion gradient. Dill had consistently the lowest saturation at all stations where arterial blood was obtained, and Christensen had one of the highest. For two stations the results are summarized in table 3. It is clear that the higher saturation in Christensen's blood is largely due to the fact that he maintains an alveolar pO_2 sufficiently high to more than overcome the handicap of a high ΔpO_2.

From the illustration given in table 3 one might conclude that the partial pressure of oxygen in alveolar air plays the major rôle in determining the saturation of arterial blood. This conclusion has been tested by plotting in figure 1 the per cent saturation as a function of alveolar pO_2 for all subjects at each of the five stations. There are also included data on 6 Peruvian students after an 8-hour journey from sea level to 4.75 km., as reported by Barron, Dill, Edwards and Hurtado (18), and on 4 members of the Leadville party (12). The relation is good enough so that with few exceptions the oxygen saturation can be predicted from the alveolar pO_2 with an error of less than ±5 per cent. It is particularly noteworthy that the two men who had resided for years at 'Quilcha and from whom alveolar air samples were obtained were in the same class with ourselves. Although alveolar air samples were not obtained from the other residents convincing evidence that secretion is not acquired after complete adaptation is found in the fact that the average arterial oxygen saturation for the 7 residents studied was exactly the same as that of our party, *viz.*, 76.2 per cent (see table 2B and 2C).

TABLE 3

Conditions for oxygen transfer in two subjects

	MONTT, 4.70 *km.*		'QUILCHA 5.34 *km.*	
	D*	C	D	C
Art. HbO_2, %	72.6	82.8	65.4	76.3
Alv. pO_2, *mm. Hg*	44.1	52.4	37.4	45.8
ΔpO_2, *mm. Hg*	+2.8	+7.0	−0.2	+4.1
pH_e	7.46	7.51	7.40	7.45
pO_2 at half saturation and $pH_e = 7.1$	29.9	30.7	29.9	29.5

* Mean of two experiments.

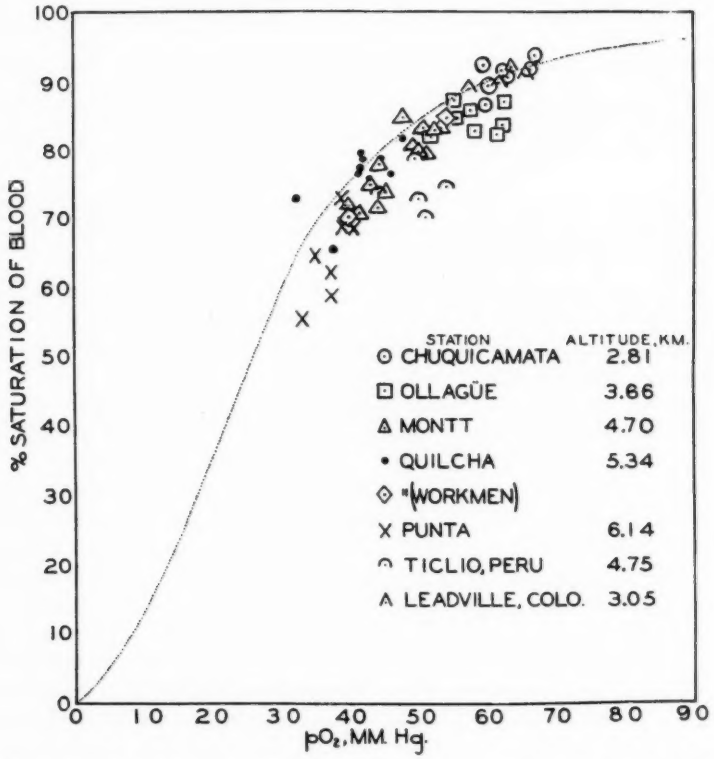

Fig. 1. The points show the relation between arterial saturation and alveolar pO_2. The dotted line is the oxygen dissociation curve of normal human blood at sea level. If the arterial pO_2 is assumed to be 4 mm. less than the alveolar pO_2 the arterial saturation can be predicted within ±5 per cent in five-sixths of the cases.

While it is true that the alveolar pO_2 is of major importance in determining the uptake of oxygen in the lungs, other factors deserve some mention. It has been noted above that the Cerro de Pasco party concluded that the oxygen dissociation curve shifts to the left, thus facilitating the uptake of oxygen. In our experience, if any change occurs, it is in the opposite direction. Hall (19) with his spectrocomparator found no change in the curve for diluted blood at constant pH. Keys, Hall and Barron (14) found the curve for whole blood is shifted to the right at constant pH. It is to be noted in table 2 that the arterial serum of the members of our party was usually more alkaline than at sea level. Normal distribution of CO_2 between cells and serum was observed and it may be deduced, therefore, that cells, also, are slightly more alkaline during acclimatization to high altitude. The net effect is to leave the dissociation curve for arterial blood virtually unchanged except at the highest stations where it is 2 or 3 mm. to the right.

The impressive success of Harrop (20) in predicting the incidence of mountain sickness of members of the Barcroft party from his measurements of the diffusion coefficient suggests that there may be significant facts about diffusion not revealed by our methods. There certainly was no simple relation in the members of our party between mountain sickness and either ΔpO_2 or arterial saturation. Hall, who had a high saturation and a low ΔpO_2 suffered most from mountain sickness. Talbott with a low ΔpO_2 but with a low saturation had very little mountain sickness until he reached the highest station where he had an acute attack of great severity.

Another observation was made which indicates that the occurrence of mountain sickness does not depend closely on the oxygen saturation of arterial blood. In several individuals arterial blood was drawn soon after arrival at a station and again a week or ten days later. Usually little change was found to occur in saturation although acclimatization had been going on in the meanwhile. In the case of Dill at Montt the saturation on the day of arrival was 73.7 per cent and 9 days later 71.6 per cent. On the second day after arrival he had typical mountain sickness, but from that time on remained free of symptoms despite the fact that he had the lowest saturation recorded at this station. At the next station, 640 meters higher, his arterial saturation dropped to 65.4 per cent, but acute symptoms of mountain sickness did not recur. Our observations agree with those of Haldane that blueness of the lips, common in newcomers to high altitude, is associated with mountain sickness and is less evident or absent in acclimatized subjects. However, it is not correct to deduce the saturation of arterial blood from the color of the lips. The work of Grollman (21) indicates that circulatory changes of the first order are going on during acclimatization and our observations are confirmatory; during the

first days at high altitude the resting minute volume may be doubled. The changing color of the skin may be more closely dependent on cardiovascular conditions than on arterial saturation.

CONCLUSION

The opinion of Krogh, Barcroft and others that diffusion can account for the transfer of oxygen in the lungs is fully confirmed. At high altitudes the partial pressure of oxygen in arterial blood, measured by referring its percentage saturation with oxygen to the dissociation curve of the same specimen of blood, is approximately equal to that in alveolar air.

The oxygen saturation of arterial blood does not necessarily increase during acclimatization. Men who have lived for years at 5.34 km. have saturations ranging from 67.6 to 84.6 per cent. Our own values after only a few days at this altitude were within the same range and had the same average value.

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CALCIUM CHANGES IN THE PLASMA RESULTING FROM BRIEF SEVERE WORK AND THE QUESTION AS TO THE PERMEABILITY OF THE CAPILLARIES TO CALCIUM

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In human subjects brief severe work produces increases in both hemoglobin and plasma protein concentrations which are best interpreted as the result of a loss of water from the blood to the tissues (Dill, Talbott and Edwards, 1930; Keys and Taylor, 1935). Immediately following running to exhaustion in 1 minute the oxygen capacity has been found to be increased over rest by 3.9 to 12.8 per cent while the plasma protein had risen by 7.7 to 17.3 per cent and analysis of these results by means of colloidal osmotic pressure data indicated an average loss of about 400 cc. of water from the blood of these subjects. In these experiments the analyses indicated only a small gain to the circulating blood in new red cells (about 20 cc. on the average); at the same time it appeared that a small amount of protein (a total of the order of 5 gm.) was lost from the plasma (Keys and Taylor, 1935).

It may be assumed that most, if not all, the water lost in these experiments was filtered out through the capillary membranes. Since it is generally held that these membranes are freely permeable to all crystalloids, it is desirable to inquire whether the water lost is accompanied by proportional amounts of mineral salts. Margaria (1930) found that the total osmotic pressure of the blood may be increased by as much as 9 per cent in experiments of this kind, but a large part of this increase may be ascribed to the products of metabolism. We have found (unpublished experiments) only small changes in the total mineral concentration in the blood in similar experiments.

The behavior of calcium under these conditions should be of particular interest because it should throw light on the diffusibility of calcium *in vivo*. It is generally assumed that the permeability of the capillary membrane is similar, qualitatively, to that of collodion or cellophane membranes. *In vitro*, it is agreed that from 40 to 70 per cent of the plasma calcium is freely diffusible through such artificial membranes and that the "non-diffusible" calcium fraction is bound to protein.[1]

[1] A survey of the literature from 1911 (Rona and Takahashi) to 1934 (Flexner) gave a mean value of 61 per cent for the fraction of the total calcium in the plasma which is diffusible or ultrafiltrable.

McLean and Hastings (1935) have developed satisfactory mass-law relationships between Ca^{++}, protein and calcium proteinate from which it appears that about 90 per cent of the diffusible calcium in human serum may be considered to be Ca^{++}. This is a somewhat larger figure than that obtained by Neuhausen and Marshall (1922) but in any case it appears that most of the "diffusible" calcium is in the form of simple calcium ions.

In the present paper the attempt is made to evaluate the permeability properties of the capillary membranes to calcium by the production of filtration *in vivo* by brief severe work.

METHODS. The general procedure was identical with that used by Keys and Taylor (1935). Healthy young men served as subjects. After resting in bed for at least 30 minutes a blood sample was taken, the subject then ran on a motor-driven treadmill for a period of 1 minute at a rate near the limit of his ability, and a second blood sample was taken within a minute after the end of work. Subsequent blood samples were taken while the subject rested in bed. In some preliminary experiments work to exhaustion in about 1 minute was performed on a rowing machine and in standing running.

All blood samples were taken without stasis from an arm vein. Heparin was used to prevent clotting and the calcium content of the heparin was allowed for in the analyses. Plasma was separated within the hour and thereafter kept at 0°C. until analysed. Analyses were made in duplicate, calcium being determined by the method of Clark and Collip (1925) and protein by the macro-Kjeldahl method.

Change of calcium concentration following work. Preliminary experiments showed that brief severe work of the type described results in a marked increase in calcium concentration in the plasma and that the original resting level is regained in about an hour or somewhat less. Figure 1 illustrates a typical experiment on the treadmill; the maximum [Ca] is attained about a minute after the end of work and thereafter the values decrease more or less along a logarithmic line until the resting level is regained.

The results in table 1 show that in different subjects after different kinds of work there are increases in calcium concentration which would imply extreme hemo-concentration if they result simply from retention of the "non-diffusible" fraction of the plasma calcium. The values in the bottom row of figures in table 1 are calculated on the basis of the *in vitro* figure that 60 per cent of the calcium is readily and completely diffusible. Obviously these values are next to impossible and they greatly exceed the maximum hemo-concentration values calculated for comparable work (Keys and Taylor, 1935).

It must be concluded that in these abrupt translocations of water from blood to tissue either the calcium fraction ordinarily considered diffusible

is not freely diffusible, or that large amounts of new calcium enter the plasma. Evidence on these alternatives may be gained by a simultane-

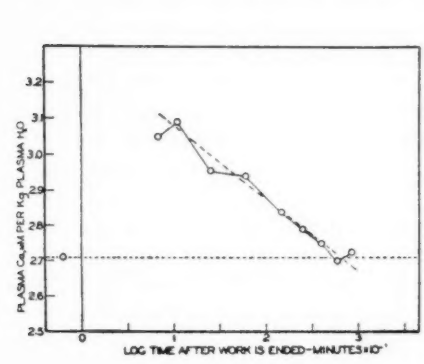

Fig. 1

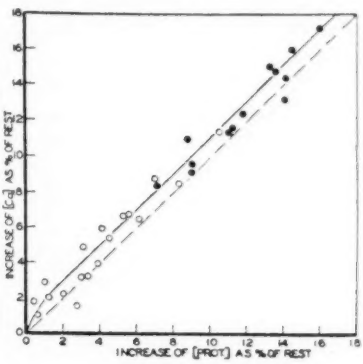

Fig. 2

Fig. 1. Plasma calcium concentration in recovery from running to exhaustion in 1 minute. The dotted line is the resting level. The recovery samples were taken after work at the following times (in minutes): 0.7, 1.1, 7.8, 15, 30, 60, 90.

Fig. 2. The relation between the changes in plasma calcium and protein concentrations following work to exhaustion in 1 minute. Solid circles represent points obtained from blood samples drawn about a minute after the end of exercise, open circles are points obtained at later stages of recovery. The broken line is the line of equal proportion, the solid line is the apparent "best fit" to the points.

TABLE 1

The effect of brief severe work on the plasma calcium level

Duration of work from 40 to 80 seconds. All values in mM. Ca per kgm. plasma water.

	KIND OF WORK					
	Treadmill		Rowing		Standing Running	
	Subject					
	R. B.	A. K.	A. S.	F. H.	A. S.	A. K.
[Ca], rest	2.58	2.62	2.83	2.77	2.92	2.71
[Ca], 1 min. after work	2.82	2.96	3.28	3.02	3.27	3.12
Increase in [Ca]	9.5%	13%	16%	9%	12%	15%
Degree of plasma concentration implied if only 40% of total Ca non-diffusible	(24%)	(32%)	(40%)	(23%)	(30%)	(38%)

ous study of plasma protein and calcium under these conditions. Figure 2 summarizes experiments in which this was done. The calcium and protein concentration changes are closely parallel, though there is a consistent

tendency for the calcium to increase to a slightly greater extent than the protein. Accordingly, almost all the calcium concentration change can be explained by water loss from the plasma with complete retention of all the calcium; i.e., under these conditions *none* of the calcium may be diffusible.

This apparent impermeability of the vascular membranes to calcium is not necessarily transitory; the parallelism between change in protein and calcium concentrations is maintained throughout the entire recovery period, i.e., up to 1 hour. A detailed experiment is given in table 2 (note also the recovery points in fig. 2).

Physiological implications. Heretofore, impermeability of the capillary walls to the "diffusible" fraction of calcium has not been suggested for a variety of reasons: 1, it is a general belief that all crystalloids diffuse

TABLE 2

Parallelism between calcium and protein concentration in the plasma in hemo-concentration and recovery from brief severe work

Concentrations per kgm. plasma water. Subject D. C., running 1 minute on treadmill at 7° angle and speed of 11.5 miles per hour.

TIME AFTER END OF WORK	[Ca]	[Ca] CHANGE FROM REST	PROTEIN	PROTEIN CHANGE FROM REST
minutes	*mM*	*per cent*	*grams*	*per cent*
Rest	2.77		7.71	
0.7	3.09	+11.6	8.57	+11.2
2.0	3.08	+11.4	8.52	+10.5
7.2	2.95	+6.5	8.19	+6.1
15.0	2.88	+4.0	8.01	+3.9
30.0	2.86	+3.2	7.94	+3.0
60.0	2.82	+1.8	7.75	+0.4

freely through the capillary membranes and that these latter closely resemble collodion or cellophane membranes with regard to qualitative permeability; 2, the "diffusible" calcium concentration in the plasma normally corresponds closely with the [Ca] in cerebro-spinal fluid (Depisch, 1926; Lickint, 1926; Cantarow, 1929; Barrio, 1932; McLean and Hastings, 1935), and in edema fluid containing little protein (Salvesen and Linder, 1923; Gollwitzer-Meier, 1925; Schade et al., 1926) 3, in synovial fluid (Cajori, Crouter and Pemberton, 1926), in cervical lymph (Heim, 1933) and in ordinary serous exudates and transudates (Gilligan, Volk and Blumgart, 1934) the [Ca] values approach those of plasma when allowance is made for the differing protein concentrations on the basis of protein "binding" of Ca; 4, introduction of additional calcium into the blood stream produces only a transient rise in plasma [Ca] (Clark, 1920; Jansen, 1924); 5, calcium is excreted by the kidney.

In view of the present results some of these arguments may be reconsidered briefly. When this is done, their strength largely disappears.

The agreement between plasma and cerebro-spinal fluid values for [Ca] does not hold when the plasma [Ca] level is altered nor do the "diffusible" [Ca] values in C. S. fluid correspond closely to those in the plasma (Leicher, 1922; Greenberg and Ballard, 1928; Greenberg, 1930; Hertz, 1930). In general, the C. S. fluid [Ca] remains constant in the face of large changes in plasma [Ca] (Merritt and Bauer, 1931). It is not unlikely that similar relations hold with the other body fluids.

The introduction of additional calcium into the blood stream produces a rise in plasma calcium which is discernible for several hours at least; if the amount of added calcium is at all large hemo-dilution is produced (similar to the result of colloid injection) (Clark, 1920). Calculations from the published data of these experiments show that at most only a fraction of the added calcium is distributed to the tissue fluids.

The ability of the kidney to excrete calcium is apparently very limited. When calcium is injected into the blood most of it is excreted in the feces (Dadlez, 1926) and normally not even all the calcium resulting from endogenous metabolism is excreted by the kidney (Wellman, 1907). This suggests that even the glomerular membrane is not freely permeable to calcium.

It seems reasonable to conclude, then, that the capillary membranes are permeable only with great difficulty even to the "diffusible" form of plasma calcium. Since there is good evidence that most, if not all of the "diffusible" calcium consists of Ca^{++} ions (cf. e.g., McLean and Hastings, 1935), it follows that these membranes are by no means freely permeable to Ca^{++}, though very slow equilibration may take place.

The consequences of this retention of calcium must be considerable. At least the Ca^{++} ions of the "diffusible" (*in vitro*) calcium fraction must exert an osmotic force similar to that exerted by the colloids. In the resting animal in equilibrium or in a steady state this osmotic contribution of calcium to the plasma will be more or less balanced by the effect of the calcium on the outer side of the capillary membrane, but as soon as any force tends to distort the blood volume an osmotic force is set up in opposition.

The general order of the total available osmotic pressure of the calcium may be estimated on the basis that, on the average, about 60 per cent of the total calcium is "diffusible" (i.e., not bound by protein) and of this about 80 per cent represents Ca^{++}. Under no circumstances, however, could more than a fraction of this "available" osmotic pressure become effective, the amount being determined by the extent to which the plasma volume is altered. A quantitative approximation, however, can be calculated for the present experiments.

It is necessary to make several assumptions, none of which is unreasonable: 1, in rest the total concentration of ions not bound by protein is the same per unit of water in the plasma and in the adjacent tissue fluid; 2, in rest the concentration of calcium ions per unit of water is the same in the plasma and in the adjacent tissue fluid; 3, in all conditions an approximate Donnan equilibrium will hold at the vascular membranes. The essential data are given in table 3 for the average of the present experiments.

For the present purposes it suffices to treat the fixed anions and cations other than calcium as monovalent and to assume activities of unity throughout. When this is done, the following relations hold:

The subscripts p and t indicate plasma and tissue fluid (peri-capillary fluid plus red cell fluid) respectively, subscripts r and w indicate rest and end of work respectively. The square brackets indicate concentrations in milli-equivalents per kilogram of water; *Vol.* indicates fluid volume. B^+ designates total cations other than Ca^{++} not bound to protein, A^- represents total anions.

(1) (electrical neutrality), $[A^-]_{wt} = [Ca^{++}]_{wt} + [B^+]_{wt}$

(2) (Donnan equilibrium), $[B^+]_{wp} \cdot [A^-]_{wp} = [B^+]_{wt}\,[A^-]_{wt}$

(3) (from (1) and (2)), $[B^+]_{wt} = \dfrac{-[Ca^{++}]_{wt} + \sqrt{[Ca^{++}]^2_{wt} + 4[B^+]_{wp} \cdot [A^-]_{wp}}}{2}$

(4) (total volume constant), $Vol._{rp} + Vol._{rt} = Vol._{wp} + Vol._{wt}$

(5) $[Ca^{++}]_{rp}\ Vol._{rp} + [Ca^{++}]_{rt}\ Vol._{rt} = [Ca^{++}]_{wp}\ Vol._{wp} + [Ca^{++}]_{wt}\ Vol._{wt}$

(It is not strictly true that total Ca^{++} is constant, but the error is negligible here.)

(6) $Vol._{wp} = \dfrac{[Ca^{++}]_{rp}\ Vol._{rp}}{[Ca^{++}]_{wp}}$ (like 5)

(7) $[Ca^{++}]_{wt} = \dfrac{[Ca^{++}]_{rp} + Vol._{rt}\,[Ca^{++}]_{rp} - Vol._{wp}\,[Ca^{++}]_{wp}}{Vol._{wt}}$

From equations (1), (3), (6) and (7), the values given in table 4 have been calculated for the range of $Vol._{rt}/Vol._{rp} = 1$ to 4. In table 5 the corresponding effective osmotic pressure values are given. They range from about 30 to 60 mm. H_2O, or from 10 to 20 per cent of the total normal colloidal osmotic pressure and accordingly represent very significant additions to the force tending to restore the blood volume to normal.

It should be noted that the large changes in the total vapor pressure of the blood resulting from exercise (Margaria, 1930) do not necessarily represent effective osmotic forces; since the membranes are conceded to be easily permeable to most of the substances involved, the vapor pressure differences may represent merely concentration or diffusion gradients and might not, *per se*, restore the blood volume as would seem to be the case with calcium. On the other hand, it may be that other substances

in the plasma behave similarly to calcium and also act as "osmotic buffers." Finally, the evidence given here should be a warning against the danger of

TABLE 3

Average changes in plasma in present experiments

Concentrations per kilogram of plasma water. $[Ca^{++}]$ calculated after McLean and Hastings, 1935. $[Prot.^{-}]$ and BPs (= base bound by protein in serum) calculated after Van Slyke, Wu and McLean, 1923. Initial total base value is mean for resting blood plasma of normal subjects. pH_s values approximate.

CONDITION	[TOTAL Ca]	[TOTAL PROTEIN]	pH_s	TOTAL BASE	[PROTEIN^{-}] = BP_s	"FREE" BASE	$[Ca^{++}]$
	mM	*grams*		*m.Eq.*	*m.Eq.*	*m.Eq.*	*m.Eq.*
Rest	2.85	77.6	7.40	154.00	13.72	137.78	2.50
End of work	3.17	85.5	7.00	154.13	12.78	138.72	2.63

TABLE 4

Calculated distribution of ions between plasma and tissue fluid in rest and at end of work from data in table 5 for effective "tissue fluid" volumes from 1 to 4 times as great as the total plasma volume. Assuming equal distribution per kilogram water in rest

Concentrations in milliequivalents per kilogram water

	REST		END OF WORK				
	Plasma	Tissue	Plasma	Values for rest tissue fluid volume =			
				1.000	2.000	3.000	4.000
$[Ca^{++}]$	2.50	2.50	2.63	2.38	2.44	2.46	2.47
$[B^{+}]$	137.78	137.78	138.72	138.84	138.82	138.81	138.81
$[A^{-}]$	140.28	140.28	141.35	141.22	141.26	141.27	141.27
Volume	1.000	1.000	0.951	1.049	2.049	3.049	4.049
	1.000	2.000	0.951				
	1.000	3.000	0.951				
	1.000	4.000	0.951				

TABLE 5

Effective osmotic pressures developed solely from retention of calcium

Data from table 6; Δ values are excess in plasma over tissue fluid.

	RATIO, $\frac{Vol._{ts}}{Vol._{tp}}$ =			
	1	2	3	4
Δ of Σ m Eq./L	0.26	0.18	0.16	0.15
Δ O. P., mm. Hg	4.46	3.06	2.72	2.55
Δ O. P., mm. H_2O	59.8	41.3	36.7	34.4

drawing inferences about *in vivo* conditions from experiments with artificial membranes, all of which so far seem to be completely and readily permeable to calcium ions.

SUMMARY

After brief severe exercise there is a marked rise in plasma calcium concentration, the maximum change appearing about a minute after the end of work and amounting to as much as 17 per cent of the resting value. After the peak is reached the plasma [Ca] declines approximately along a logarithmic line until the resting level is reached at from 30 to 70 minutes after the end of work.

These changes in [Ca] are closely parallel to changes in plasma protein concentration, the per cent rise in [Ca] generally exceeding very slightly the rise in protein concentration.

It is concluded that these concentration changes result almost entirely from exchanges of water between blood and tissue and that under these conditions even the so-called "diffusible" calcium is restrained from diffusion by the capillary membranes to the same extent that plasma proteins are restrained.

It follows that under these conditions the "diffusible" calcium will be osmotically active in the blood stream and the net osmotic pressure developed in these experiments by the "diffusible" calcium is calculated to be of the order of 30 to 60 mm. of water.

The rôle of the "diffusible" calcium as an "osmotic buffer" in these conditions is stressed and it appears that this force must be taken into account in calculating the balance of forces controlling fluid movement from blood to tissue.

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ON THE EXCRETION OF SKIODAN, DIODRAST AND HIPPURAN BY THE DOG[1]

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Evidence, recently obtained in the study of the excretion of inulin, (Richards, Westfall and Bott, 1934; Shannon, 1935a), strengthens the belief that in dogs ingested or injected creatinine is not secreted into the urine by the renal tubule but is excreted wholly by glomerular filtration. Reabsorption of creatinine from the tubule appears to be minimal and hence there is at present good justification for regarding the plasma clearance of creatinine in dogs as a minimal indirect measure of the volume of glomerular filtrate. On the other hand it seems certain from the experiments of Marshall and Vickers (1923), Marshall (1931), Mackay and Oliver (1930) that the dyes, phenol red and neutral red, are in significant part secreted by the dog's tubule. Evidence is lacking which makes it possible to picture the processes of tubular secretion or to define the characteristics of a substance which determine that the tubule shall secrete it. Hence it seems to be important to seek other substances than the dyes mentioned whose rate of elimination is too great to be explained by filtration. Since the plasma clearance of creatinine is higher than that of other normal urinary constituents it is obvious that search for such substances must be made among those which are foreign to the animal body.

Recently introduced into clinical urology are certain organic iodine compounds which, by reason of their opacity to x-ray and of the high concentration in which they appear in urine, have been found useful in urography. The rapidity of their excretion led to the thought that in this group of compounds might be found some which are secreted by the tubules. The experiments described below indicate that this is true of diodrast and hippuran, not of skiodan.[2]

[1] The expenses of this investigation were defrayed in large part from a grant by the Commonwealth Fund. A preliminary report by Elsom, Bott and Landis appeared in the Proc. Soc. Exper. Biol. Med. **32**: 77, 1934.

[2] Diodrast is 3:5-diiodo-4-pyridon-N-acetic acid diethanolamine (50 per cent iodine); hippuran, sodium ortho-iodohippurate (38.3 per cent iodine); skiodan, mono-iodo-methane sulphonate of sodium (52 per cent iodine).

METHODS. The subjects were unanesthetized female dogs, kept on a mixed diet. Urine specimens were collected by catheter, especial care being taken to empty the bladder completely. Blood samples were taken from the external jugular vein.

In one group of experiments an initial dose of creatinine (1 to 2 grams) and of the iodine compound (0.38 to 3.88 grams I), was given intraperitoneally or intravenously 10 or more minutes before the beginning of the first clearance period, and followed during the periods by the slow, constant infusion of a solution of the two substances into the saphenous vein. The periods were approximately 30 minutes long and blood was taken at the beginning and end of each.

In the second group the two substances were given as a single intravenous injection 4 to 11 minutes before the beginning of the first clearance period (creatinine, 3 to 5 grams; the iodine compound, 0.52 to 6.25 grams I). The periods were approximately 15 minutes in duration, blood being taken exactly at the midpoint of the period.

These differences in procedure were without apparent effect upon the results, hence the 2 groups are not considered separately.

Clearances were calculated by the usual formula, $C = \frac{U \times V}{B}$ where U is the urinary concentration, V the urine volume and B the plasma concentration.

Chemical methods. Blood analyses were made on plasma. Lithium oxalate was used as anticoagulant. The blood was centrifuged within 5 minutes after collection to avoid errors arising from the diffusion of the iodine compound and creatinine from the plasma into the erythrocytes. Plasma and urine were analyzed for creatinine by the Holten and Rehberg (1931) modification of the Folin method. In a few experiments plasma filtrates were prepared by the method of Steiner, Urban and West (1932) and were analyzed for creatinine by Folin's method. Plasma and urine were analyzed for iodine by the method of Leipert (1933) with slight modifications. The method consists in 1, the conversion of iodine into iodate by digestion with chromo-sulphuric acid, with ceric sulphate as catalyst; 2, reduction of iodate to iodine, which is transferred by steam distillation into an alkaline absorbent (KOH); 3, oxidation of the absorbed iodine by bromine; 4, removal of the excess bromine, and 5, thiosulphate titration of the iodine liberated from added potassium iodide. When the iodine content of the sample was expected to be lower than 0.1 mgm. the alkali containing the distilled iodine was evaporated to a small volume to facilitate the iodine titration. Bromine was always converted to tribromphenol instead of being removed by steam.[3]

[3] In this detail and in the choice of KOH as absorbent our procedure differed from that of Leipert.

The accuracy of the method is indicated by the following summary of the results of single analyses of 1 cc. samples of water, serum, urine, whole blood and 1 gram samples of kidney pulp to which the various iodine compounds were added.

AMOUNT OF IODINE IN SAMPLE	NO. OF ANALYSES	AVERAGE ERROR	MAXIMUM ERROR
mgm.		*per cent*	*per cent*
0.50 –1.00	15	0.6	1.7
0.100–0.500	17	1.2	3.0
0.020–0.100	8	2.3	4.7
0.005–0.020	16	4.1	10.0

All analyses in the clearance experiments were made in duplicate. For iodine analyses 1 cc. plasma samples were used, containing as a rule more than 0.1 mgm. of iodine. Urine was usually diluted, 1 cc. samples being analyzed.

TABLE 1

Comparison of skiodan and creatinine clearances in the normal dog

5/3/1935. Dog 1; 14 kgm.; 2475 cc. of water by mouth in the 5 hours immediately preceding the experiment; 3 grams of creatinine, 12 grams of skiodan intravenously at 2:10.

TIME	URINE FLOW	PLASMA		CLEARANCES		CLEARANCE RATIO $\frac{\text{IODINE}}{\text{CREATININE}}$
		Iodine	Creatinine	Iodine	Creatinine	
	cc. per min.	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	
2:16–2:30	8.29	103.0	35.5	46.5	52.0	0.89
2:30–2:45	5.07	78.7	25.9	50.6	56.0	0.90
2:45–3:00	3.33	63.7	22.2	49.8	50.2	0.99
3:00–3:19	2.71	50.3	16.7	48.6	51.9	0.94
3:19–3:35	2.50	41.0	15.1	48.7	50.6	0.96
3:35–3:52	2.00	34.1	13.3	48.2	51.7	0.93

RESULTS. *Comparison of the clearances of skiodan*[4] *and creatinine.* Thirty-three clearances were measured in 8 dogs. A typical experiment is outlined in table 1. The results of the entire group are charted in figure 1a, the ratios, $\frac{\text{skiodan iodine clearance}}{\text{creatinine clearance}}$, being plotted against plasma iodine

[4] It is assumed throughout this paper that the iodine compounds studied exist in plasma wholly in filtrable form. The assumption is based on the fact that ultrafiltration through cellophane membranes (no. 450) of horse serum, to which the different compounds had been added in widely different amounts, yielded filtrates having the same iodine content as the original solutions.

concentration. The skiodan clearances were lower than those of creatinine by an average of 11 per cent; they were independent of the plasma iodine concentration over a range of 3.2 to 114.4 mgm. per cent, and also of the rate of urine formation between the limits of 0.25 to 8.3 cc. per minute.

Comparison of the clearances of diodrast and creatinine. Thirty-four comparisons in 7 dogs were made. A relationship very different from that described for skiodan exists between the excretion of diodrast and creatinine. The data from a representative experiment (table 2) show that the magnitude of the diodrast clearance was strictly dependent upon the concentration of this substance in the plasma. At high levels it approximated

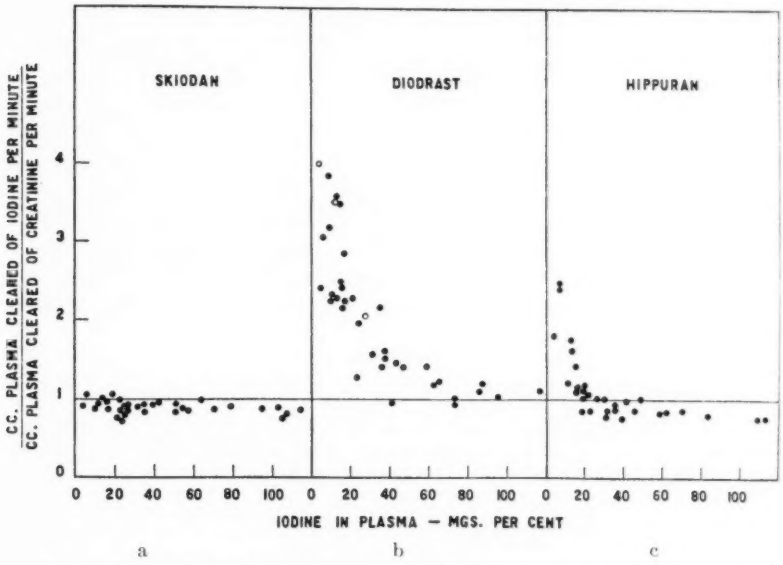

Fig. 1. Comparison of the clearances of skiodan, diodrast and hippuran with simultaneous clearances of creatinine in the dog.

that of creatinine, but was 4 times as great when the plasma iodine concentration approached zero. In figure 1b the ratios of $\frac{\text{diodrast iodine clearance}}{\text{creatinine clearance}}$ are plotted against the plasma iodine concentration. The variation in the ratios with changing plasma iodine level were due to changes in the diodrast clearance rather than in that of creatinine. It will be observed that even at the highest plasma iodine levels studied identity between the diodrast and creatinine clearances was not consistently obtained.

The 3 open circles in figure 1b represent the results of an experiment in which the animal had received a daily subcutaneous dose of 1.0 gram of

purified phlorhizin for 12 days prior to these observations. The D:N ratio (3.64) indicated complete phlorhizinization. The usual relationship between the creatinine and diodrast clearances was observed.

Comparison of the clearances of hippuran and creatinine. As in the experiments with diodrast a reciprocal relationship between the plasma iodine concentration and the magnitude of the clearances was observed in the

TABLE 2

The comparison of diodrast and creatinine clearances in the normal dog

4/25/34. Dog 2; 15.3 kgm.; 400 cc. water by mouth at 9:55; 1.5 grams creatinine intraperitoneally at 10:25; 7 grams diodrast intravenously at 10:45. Intravenous infusion of a solution containing 6.0 per cent diodrast, 0.4 per cent creatinine during first clearance period only. Rate of injection 1.5 to 1.0 cc. per minute.

TIME	URINE FLOW	PLASMA		CLEARANCES		CLEARANCE RATIO IODINE / CREATININE
		Iodine	Creatinine	Iodine	Creatinine	
	cc. per min.	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	
10:57–11:25	3.93	95.6	15.6	45.2	43.5	1.04
11:25–11:55	3.73	72.5	14.2	49.6	49.2	1.01
11:55–12:25	0.70	42.6	11.1	56.3	38.2	1.47
12:25–12:57	0.42	24.0	8.5	96.8	49.0	1.97

TABLE 3

Comparison of hippuran and creatinine clearances in the normal dog

Dog 3; 13.2 kgm. Representative periods from 3 experiments which showed wide variations in plasma iodine concentration. Creatinine and hippuran injected together 21, 34 and 127 minutes before these observations. No intravenous infusion.

DATE (1935)	URINE FLOW	LENGTH OF PERIOD	PLASMA		CLEARANCES		CLEARANCE RATIO IODINE / CREATININE
			Iodine	Creatinine	Iodine	Creatinine	
	cc. per min.	*min.*	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	
5/9	3.27	15	45.7	52.3	53.4	61.0	0.87
	5.20	15	35.0	41.8	55.1	61.4	0.90
6/14	1.19	10.5	15.4	35.1	73.5	66.5	1.10
2/26	4.76	33	7.1	17.2	170.0	68.2	2.50

study of hippuran (table 3). At low plasma iodine levels the hippuran clearance was 2.5 times that of creatinine. In contrast to the behavior of diodrast, however, it will be observed in figure 1c that only when the plasma iodine remained below 20 mgm. per cent was the hippuran clearance greater than that of creatinine. When it was above 20 mgm. per cent the

hippuran clearance fell approximately 12 per cent below the creatinine clearance.

The decrease in both hippuran and diodrast clearances caused by high plasma iodine concentrations continued only as long as the concentration remained high. The ability to excrete both substances more rapidly than creatinine returned simultaneously with a fall in the plasma iodine level. In the experiment shown in figure 2 a single large dose of hippuran was injected intravenously and its clearance measured for 6 consecutive 15 minute periods thereafter. The clearance remained low until the plasma iodine concentration had fallen to approximately 20 mgm. per cent, when a striking increase in the clearances occurred.

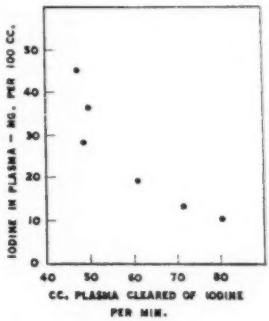

Fig. 2. Showing the relationship of the plasma iodine concentration to the iodine clearance after a single intravenous injection of hippuran into a dog.

Comparison of the clearances of sodium iodide and creatinine. The contrast between the behavior of these organic iodine compounds and the inorganic iodides was observed in an experiment in which the clearance of sodium iodide was compared to that of creatinine (table 4).

TABLE 4

Comparison of the clearances of sodium iodide and creatinine in the normal dog

6/13/34. Dog 4; 14.4 kgm.; 400 cc. water by mouth at 1:23; 1.5 grams creatinine intraperitoneally at 1:40; 2.5 grams of sodium iodide intravenously at 2:03. Intravenous infusion of a 5 per cent solution of sodium iodide during first period only. Rate of injection 1.3 to 1.0 cc. per minute.

TIME	URINE FLOW	PLASMA		CLEARANCES		CLEARANCE RATIO IODINE / CREATININE
		Iodine	Creatinine	Iodine	Creatinine	
	cc. per min.	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	
2:20–2:53	0.76	61.4	13.5	5.8	45.4	0.128
2:53–3:23	0.33	66.7	11.7	7.2	49.5	0.145
3:23–3:56	0.60	59.2	9.5	13.2	62.5	0.211
3:56–4:32	0.39	54.2	7.6	9.6	51.4	0.187
						Av. 0.168

Discussion. If the belief stated in the first paragraph of this paper is correct, that the volume of glomerular filtrate in the dog is at least as great as the plasma clearance of creatinine, it follows that in the excretion of another urinary constituent whose clearance is less than that of creatinine

there is no necessity for assuming the operation of any other process than that of filtration. With this assumption the conclusion is justified that all of the urinary skiodan leaves the blood in the glomerular filtrate, and that of this, on the average 10 per cent is reabsorbed.

In the excretion of diodrast, on the contrary, we are obliged to assume that secretion by the tubule occurs to an extent which depends upon the concentration of diodrast in plasma. This assumption is strengthened by calculations of the percentage of plasma which would have to be filtered at the glomeruli to yield a volume of filtrate which contained the amount of diodrast eliminated when the plasma concentration is low. At a plasma iodine concentration of 9.6 mgm. per cent a 12 kgm. dog excreted 18.6 mgm. of diodrast iodine per minute. If one assumes that the animal had the maximal renal blood flow observed in the unanesthetized dog, 7.4 cc. per gram of kidney per minute (Van Slyke et al., 1934), then, estimating kidney weight from surface area (Van Slyke et al., 1934), 350 cc. of blood flowed through the kidneys in a minute. Assuming that 60 per cent of the blood was plasma, 210 cc. of plasma containing 20.2 mgm. of iodine flowed through the kidneys per minute. For this volume of plasma to yield by filtration 18.6 mgm. of iodine requires the incredible assumption that over 90 per cent of the plasma water was filtered into the glomerular capsules.

Hippuran occupies a position between skiodan and diodrast in its behavior. Tubular secretion is required to account for its clearance at low plasma iodine levels, while at high levels, on the contrary, its reabsorption or back diffusion must be postulated.[5]

The clearance of both diodrast and hippuran varies inversely with their concentration in the plasma. In this respect the excretion of these substances in the dog resembles that of phenol red in the frog and dog (Marshall and Vickers, 1922; Marshall and Crane, 1924; Marshall, 1931; Shannon, 1935b), neutral red in the rabbit (Mackay and Oliver, 1930), creatinine in the dogfish (Shannon, 1934) and in man (Shannon, 1935c). Our findings, therefore, may be regarded as additional support of the view that this relationship is a valid criterion of tubular secretion.

SUMMARY AND CONCLUSIONS

Clearances of three organic compounds of iodine, skiodan, diodrast and hippuran, have been compared to that of creatinine in the dog. The clearance of skiodan is independent of the concentration of this substance in the plasma, and is consistently less than that of creatinine by approximately 10 per cent. The clearances of diodrast and hippuran are strictly dependent

[5] It may be of some future importance to note that the striking difference between the renal behavior of skiodan and that of diodrast in dogs is not found in toadfish. Preliminary experiments indicate that both substances are excreted rapidly and in high concentration in urine from the aglomerular kidney.

upon the concentration of these substances in the plasma. At high plasma iodine levels their clearances approximate that of creatinine, at low plasma iodine levels they exceed that of creatinine several fold. The results are interpreted to indicate that the elimination of skiodan may be attributed to glomerular filtration alone, while the excretion of diodrast and hippuran requires the postulation of tubular secretion.

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SKELETAL CHANGES IN THE RAT INDUCED BY A RATION EXTREMELY POOR IN INORGANIC SALTS[1]

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Several studies have been made to determine the nature and extent of the physiological adjustment made by rats subsisting on a diet extremely poor in inorganic salts. It has been found that growth ceases, and that body weight remains essentially stationary over a relatively long period, while growth in length of the body and of the long bones continues at a subnormal rate (22). The drain of calcium salts from the bones (5a) while they were continuing to increase in size made it seem desirable to determine what changes were occurring in such bones. Accordingly, analyses of moisture, ash and organic residue, as well as measurements of length and diameter were made on several of the long bones of rats consuming the low-salt ration for periods of 3, 6 and 12 weeks. Inasmuch as the chemical changes induced in the bones of the rats were profound, a study was made to determine to what extent recovery to normal chemical composition occurred, when rats kept on the low-salt diet were subsequently "realimented" with an adequate diet for varying periods.

A study of the breaking strength of several bones was also made by the method devised by Lindsay and Howes (12). The experiment thus provided data from which to determine whether breaking strength is more closely correlated with the dimensions of long bones or with their chemical composition.

EXPERIMENTAL. Young male rats were fed the low-salt diet according to the procedure previously described (8). The data recorded here are derived from the same animals used in the study of the incisors, referred to above. Each group of experimental rats was provided with two control groups, "age controls," fed a "synthetic" diet adequate in every respect,

[1] Some of the data in this paper are taken from a dissertation presented by Miriam F. Clarke in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1933.

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[2] Davis & Geck Fellow in Surgery.

and "calorie controls," the diet of which though adequate qualitatively, was limited so that the calorie intake of this group was the same as that of the low-salt rats. The rats on the low-salt diet for twelve weeks were also provided with a third group of normal controls, much younger, but of the same body weight as the stunted rats at the end of their period of stationary weight. These were called "weight controls."

At the termination of the experimental period, the animals were killed and the bones in question removed and cleaned of adhering tissue. The femurs were weighed and then placed in a desiccator for a few days before the measurements were made. The maximum length, lateral diameter and antero-posterior diameter at the mid-point of the femur were determined by means of a vernier caliper. The length and both diameters at the point where the break was expected to occur, were determined immediately for the cleaned fibula, radius and humerus. The latter bones were then submitted to the breaking strength test.

The femurs were dried at 90°C. to determine moisture and then extracted with alcohol and ether. After the dry and fat-free weight had been determined, the bones were ashed at about 600°C. to constant weight. Ash content was calculated on the basis of fresh and of dry-extracted weight.

RESULTS. *Weight and chemical composition of the femurs.* Although the absolute weight of the femurs increased during the low-salt period, it is apparent that the relative weight decreased, inasmuch as the weight of these bones was 73, 70 and 61 per cent of the weight of the age control femurs at the end of a 3, 6 and 12 week period respectively (table 1). Although these bones are smaller than those of their age controls, the fact that they have grown at a subnormal rate is indicated by the increase of 7 per cent in weight over that of their weight controls during the 12 weeks of the low-salt regime, while the femurs of the calorie controls had increased 23 per cent in weight and those of the age controls 75 per cent. After the 12-week interval the femurs of the low-salt rats contained 14 per cent more moisture and 41 per cent less ash (based on absolute values) than the young weight controls; they contained about the same amount of moisture and 78 per cent less ash than the bones of the control rats of the same age, which were nearly twice the weight of the femurs of the low-salt rats. The increase of moisture and marked reduction of ash content in rats somewhat similarly treated have been noted (4, 11).

The ratio of ash to fat-free organic residue (A/R, table 1) shows the progressive deviation from normal of this measure of the proportion in the constituents of the bones of the low-salt rats. It is evident that the composition of growth of the bone in this group is decidedly abnormal. Smith and Schultz (18) have shown that this type of change in composition of bone also occurs in very young rats subjected to the strict re-

striction of salts in the diet. Restriction of development through quantitative underfeeding (calorie controls) did not have this effect on the A/R ratio. After realimentation of the low-salt rats, this value approached

TABLE 1
Composition of the femur

EXPERIMENTAL PERIOD	FRESH WEIGHT	MOISTURE	ASH CONTENT		A/R† RATIO
			Fresh femur	Dry-ext. femur	
Low-salt experiment					
	gm.	*per cent*	*per cent*	*per cent*	
Low-salt, 3 weeks	0.767	56.7	16.8	44.1	0.79
Age control	1.043	48.1	28.5	56.8	1.32
Calorie control	0.934	46.8	28.8	56.7	1.31
Low-salt, 6 weeks	0.857	55.7	15.1	41.6	0.71
Age control	1.226	41.9	34.5	61.2	1.58
Calorie control	1.154	40.9	33.5	60.5	1.53
Low-salt, 12 weeks	0.987	54.3	14.2	39.6	0.66
Age control	1.615	35.3	39.2	63.8	1.76
Calorie control	1.136	32.6	37.3	63.2	1.72
Weight control	0.925	51.0	25.9	55.5	1.25
Realimentation experiment					
3 + 9* low-salt	1.471	36.3	36.2	60.7	1.55
Age control	1.520	35.2	38.6	62.4	1.66
Calorie control	1.652	33.9	41.2	65.8	1.92
6 + 6 low-salt	1.262	39.2	32.6	58.5	1.41
Age control	1.520	35.2	38.6	62.4	1.66
6 + 12 low-salt	1.529	34.4	38.5	62.7	1.68
Age control	1.769	31.7	41.6	64.5	1.82
Calorie control	1.655	33.8	40.8	64.2	1.80
12 + 12 low-salt	1.558	35.8	36.4	61.9	1.63
Age control	1.976	32.0	42.7	65.7	1.92
Calorie control	1.757	32.2	39.5	65.3	1.88

* 3 weeks on low-salt diet, 9 weeks realimentation period.
† Ratio of ash to fat-free organic residue.

the normal but in no case reached it, thus indicating again the failure of the bone to attain normal composition under the conditions of the present experiment.

Following realimentation, the weight of the bones and the content of

ash increased whereas the moisture decreased. However, in the time allowed for realimentation, none of the fresh weights (except in the 3 + 9 group) and none of the values for ash and moisture returned to those of the age control. These differences are statistically valid.

TABLE 2

Average dimensions of bones in millimeters

EXPERIMENTAL GROUP	FEMUR			FIBULA			RADIUS			HUMERUS	
	Length	Diameter 1	Diameter 2	Length	Diameter 1	Diameter 2	Length	Diameter 1	Diameter 2	Length	Diameter
Low-salt 3 + 0	27.8	3.2	2.5	15.6	0.5	0.8	18.4	1.2	1.3	21.9	1.9
Age control	29.4	3.6	2.8	16.8	0.7	1.0	19.4	1.3	1.5	23.2	2.0
Cal. control	28.4	3.5	2.7	16.3	0.6	0.9	18.8	1.2	1.4	22.7	2.0
Low-salt 6 + 0	29.7	3.3	2.6	17.1	0.6	0.9	19.6	1.2	1.4	23.1	1.9
Age control	32.4	3.7	2.8	18.4	0.7	1.0	21.4	1.4	1.6	25.1	2.0
Cal. control	31.5	3.7	2.9	18.4	0.7	0.9	21.0	1.3	1.5	24.7	1.9
Low-salt 12 + 0	31.1	3.3	2.7								
Age control	36.1	4.2	3.1								
Cal. control	32.3	3.7	2.8								
Weight control	27.6	3.3	2.6								
Low-salt 3 + 9	35.2	*4.1*	*3.1*	21.1	0.7	1.1	23.2	*1.5*	*1.7*	27.2	*2.3*
Age control	34.6	3.8	2.9	20.7	0.7	1.0	23.1	1.3	1.5	26.7	1.9
Cal. control	*36.9*	4.2	3.1	*22.6*	0.8	*1.1*	*24.5*	*1.5*	*1.8*	*28.3*	2.3
Low-salt 6 + 6	*32.9*	3.8	3.0	20.2	0.7	1.0	21.9	*1.4*	*1.6*	25.9	*2.0*
Age control	34.6	3.8	2.9	20.7	0.7	1.0	23.1	1.3	1.5	26.7	1.9
Low-salt 6 + 12	35.7	4.1	3.1	*21.9*	0.8	1.1	*23.8*	1.5	*1.7*	*28.0*	2.2
Age control	37.6	4.3	3.1	22.5	0.8	1.2	25.0	1.6	1.8	29.2	2.2
Cal. control	37.5	4.1	3.1	22.6	0.8	*1.0*	24.8	1.5	*1.7*	29.1	2.3
Low-salt 12 + 12	*35.0*	*4.1*	*3.3*								
Age control	39.1	4.6	3.5								
Cal. control	38.1	*4.3*	*3.1*								

Italicized values indicate that $\frac{\text{Difference}}{\text{Standard error of the difference}}$ for experimental vs. age control group is at least 2.0.

Dimensions of bones. During the 12-week period, the femurs of the low-salt animals increased in length 3.5 mm. (13 per cent), those of the calorie controls increased 4.7 mm. (17 per cent), and those of the age controls 8.5 mm. (31 per cent). Similar differential rates of increase in length

were exhibited by the other three bones measured after the shorter experimental periods (table 2). Changes in diameters of the bones examined followed closely the other measurements.

Realimentation resulted in an increase in length and diameter to values approaching control values, and in some cases exceeding the latter. The over-compensation occurs in most of the measurements of the 3 + 9 group and in some of those of the 6 + 6 group (indicated by italics in table 2, when the difference is statistically valid) and is common to those rats

TABLE 3

Breaking strength in grams

EXPERIMENTAL GROUP	FIBULA	RADIUS	HUMERUS
Low-salt experiment			
Low-salt 3 + 0	149	624	1,536
Age control	264	1,313	3,139
Cal. control	234	1,187	3,108
Low-salt 6 + 0	135	429	1,195
Age control	294	1,621	4,279
Cal. control	332	1,262	3,659
Realimentation experiment			
Low-salt 3 + 9	*517*	*1,897*	*5,042*
Age control	306	1,604	3,761
Cal. control	*528*	*1,965*	3,454
Low-salt 6 + 6	374	1,675	3,968
Age control	306	1,604	3,761
Low-salt 6 + 12	*387*	*1,952*	*4,280*
Age control	517	2,234	5,189
Cal. control	550	*1,909*	*4,111*

Italicized values indicate that $\frac{\text{Difference}}{\text{Standard error of the difference}}$ for experimental vs. age control groups is at least 2.0.

realimented after a low-salt period and after a low-calorie period. That this accelerated growth is not merely a skeletal phenomenon, but a general one, is indicated by the fact that the body weights of the 3 + 9 rats exceed those of their controls as well as the weights of the 6 + 6 group. The acceleration in growth following short periods of delayed growth is a well-recognized phenomenon in many species (11, 16). On the other hand, following experimental periods longer than 3 weeks, the bones tend to be smaller in most cases (indicated by italics in table 2, when the difference

is statistically valid). The body weights of these rats are likewise less than those of the controls.

Breaking strength. The breaking strengths of the bones of the low-salt rats were one-third to one-half the corresponding values for the age controls (table 3). The values for the calorie controls were, with one exception, also less than those of the age controls although the difference was smaller. Following realimentation, a marked increase in breaking strength occurred. A tendency to over-compensate is observed especially in the 3 + 9 group. Comparing the breaking strengths in table 3 with the dimensions of the bones in table 2, it appears that in every case where the breaking strength was greater by a statistically valid amount, one or more of the dimensions of the experimental bones was also greater than the control values. In the 6 + 12 group of realimented rats, after both mild inanition and mineral deficiency, breaking strengths and bone measurements were, in general, less than those of the age controls.

Discussion. The extremely labile nature of bone salts is indicated by the progressive decrease in ash content of the bones of the rats subjected to the low-salt regime. It is thus apparent that the skeleton differs from the incisors, which continue to deposit mineral matter at the expense of bone under the adverse dietary conditions of this experiment (8). The difference in the per cent of ash of the low-salt incisors and of their age controls never exceeded 7.9, whereas the difference in the per cent of ash of the femurs was 24.2 after the longest period of salt restriction.

If in groups 3 + 9 and 6 + 12 the deviations from their age controls are compared on the basis of measurement (table 2), composition (table 1) and breaking strengths (table 3), a striking fact becomes apparent. At the end of the respective experimental periods, both groups fail to attain the composition of the age control by about the same amount. On the other hand, groups 3 + 9 attains dimensions definitely greater than those of the controls whereas group 6 + 12 shows measurements equal to or less than those of the controls despite the greater opportunity to recover (i.e., 12 weeks vs. 9 weeks). Correlated with the larger size of bone in group 3 + 9 is a breaking strength greater than that of the control whereas in group 6 + 12 with its smaller relative size there is a diminished breaking strength. The difference in relative breaking strengths of the bones of the two groups is thus not related primarily to per cent of ash but more closely to what might be called the architecture of the bone, i.e., the structural arrangement in which the organic constituents take an important part and in which the bone salts are deposited as the animal develops. It is apparent from the present data that the breaking strength of a bone is more closely correlated with the integrity of the organic pattern as reflected in size than with the amount of inorganic matter deposited in the bone, unless the latter is reduced to an amount much below the normal

variation of this constituent, as is seen in the low-salt groups 3 + 0 and 6 + 0. No explanation is offered for the over-compensation in growth and corresponding increase in breaking strength following realimentation after the shorter low-salt periods or after mild inanition.

The reason for the failure of the bones to grow normally following realimentation after longer periods on a deficient diet may be looked for in the structure of the bone. Examination of decalcified sections of the tibia of rats realimented after 12 weeks of salt restriction revealed marked resorption from the ends of the marrow cavity outward. Extreme irregularity in the rows of osteoblasts and bony prominences in this region, as well as a poorly calcified and irregular epiphyseal line were in evidence, even in the bones of rats realimented for a long time.[3] Permanent damage seems, therefore, to have been produced by the nutritional adjustment herein employed.

The persistent growth of the skeleton under conditions which cause the cessation of growth in body weight has been described for dogs (1), steers (21), rats (11, 15, 17, 18 and 22) and the human species (3, 7). On the other hand, it is known that the growth of bones of dogs can be limited by restriction of dietary calcium (2) or phosphorus (10). Similar effects have been observed in calves (14) and pigs (5) when mineral intake was the limiting factor. The inhibiting effect of lack of calcium and vitamin D has been studied by Templin and Steenbock (19). In young adult females kept under these restrictions for 8 months, these authors found that the growth in length of the femur was almost completely stopped and the amount of ash was reduced to 54 per cent of that of the controls of the same age at the end of the experimental period. In a shorter time, and with a much more drastic reduction of calcium as well as other mineral salts of the diet (but with adequate vitamin D), the femur of the young rats used in the present study showed slight growth, but contained only 22 per cent of the amount of ash present in the control bones of the same age.

The breaking strength of the fibula of the age controls was close to that found by McKeown et al. (13), who noted a close correlation between fibular length and fibular strength in normal rats. That some relation exists between the composition of bone and breaking strength has been suggested by Becker and Neal (4) who worked with cattle, and by Bohstedt et al. (5), who studied swine. The latter authors concluded that there was a closer correlation between per cent of bone ash and breaking strength than between the latter and the weight of the bone. Decrease of the mineral content of the gross weight of the bones occurs simultaneously in cows fed timothy hay, poor in calcium and phosphorus (9). Reduction

[3] Grateful acknowledgment is made to Dr. Caspar Burn for assistance in the histological examinations.

of the mineral intake of cows to two-fifths of the usual amount, has resulted in a disease of cattle, one of the symptoms of which is spontaneous fracture (20). The studies made with domesticated animals have shown that adding a mineral supplement or other good source of the missing elements, results in increased weight, thickness and strength of the bones. Becker and Neal (4) also refer to an over-compensation in the bone strength of cows recovering from phosphorus deficiency. So far as we are aware, the present study is the first in which an attempt was made to correlate bone dimensions other than length with bone strength.

SUMMARY

Rapidly growing male rats were restricted to an experimental ration extremely poor in salts. Although increase in body weight ceased, the length and weight of the femur, humerus, fibula and radius continued to increase slowly. During the period of salt restriction the moisture of the bone increased, the ash decreased and a marked distortion of the A/R ratio was established. Even after relatively long periods of realimentation with an adequate diet, the per cent of moisture and ash and the dimensions of the bone failed to attain values normal for the age. Breaking strength appears to be more closely correlated with organic pattern as reflected in the size of the bone than with the proportion of inorganic salts.

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THERMAL INACTIVATION OF MEDULLATED NERVE

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Observing that the temperature of thermal inactivation of nerve is similar to that of the coagulation of certain proteins and to that at which nerve begins to shorten, Alcock (1903) and Brodie and Halliburton (1904) concluded that extinction of irritability and thermal shortening are both manifestations of neuroprotein coagulation. Reinvestigation of this phenomenon showed that the shortening may be attributed in great part to oriented structures of the connective tissue, Schwann sheath and axis cylinder and that the shortening systems respond to solvating and desolvating agents much the same as other oriented tissues such as tendon (Schmitt and Wade, 1935 a, b). If thermal disorientation of organized structures in the axon causes abrupt loss of irritability as was indicated by Alcock and Brodie and Halliburton, it seemed hopeful that further investigation of the phenomenon might reveal what rôle, if any, such oriented structures play in the conduction of the impulse under normal conditions. Preliminary experiments indicated that other processes such as substrate activation and respiration might be the more immediate causes of thermal failure (Schmitt and Wade, 1934). However, since all of these processes appeared to be affected at temperatures near that of the extinction of the action potential we performed further experiments to obtain, with an accuracy as great as possible, the exact shape of the curves so as to establish, if possible, the immediate cause of failure.

The effect of temperature upon nerve processes varies considerably with the rate of heating and with the tension on the nerve during the heating. In the preliminary experiments these factors were not maintained strictly uniform. In the respiration experiments, for example, the nerves were subjected to the heating longer than in the experiments on action potential. In the experiments here reported[1] the nerves were brought to the desired temperature under uniform reproducible conditions and the various processes were measured as nearly simultaneously as possible. Thus at any given temperature action potential, shortening and respiration, or action potential, shortening and substrate activation were measured, each

[1] The experimental work described in this section was done with the technical assistance of Mr. H. Breymann.

upon the same group of heated nerves. The procedure was to subject the nerves to heating in Ringer solution at any desired temperature for an arbitrary period (15 minutes was chosen for convenience). After the period of heating the nerves were returned to Ringer solution at room temperature and the length and action potentials recorded. Thereafter, if respiration was to be measured the group of heated nerves was transferred to a Warburg differential respirometer, the unheated control nerves being transferred to a different respirometer, and the respiratory rates determined. If dehydrogenase activity was to be measured, the heated nerves, after having been returned to Ringer solution at room temperature and lengths and action potentials recorded, were cut into bits and transferred to a Thunberg tube. After the addition of the appropriate buffer solution and methylene blue, the tube was evacuated, transferred to the thermostat at 35°C. and the decolorization time noted. The unheated control group of nerves was similarly treated. To make sure that the heating was comparable in each experiment, the following procedure was adopted. A vessel was partly filled with Ringer solution and immersed or shaken in a large thermostat, the temperature of which could be regulated to better than 0.1°C. (for the special experiments on the action potential, a Warburg thermostat was used in which the temperature was constant to a few thousandths of a degree). After allowing sufficient time for the Ringer solution in the vessel to become equilibrated with that of the thermostat, the vessel was removed, the nerves plunged into the Ringer solution and the vessel replaced in the thermostat for exactly fifteen minutes. It was important in the experiments on action potentials that these precautions be adhered to strictly as a difference of a few tenths of a degree in the critical range of 43°C. makes considerable difference in the thermal effect.

The composite results are shown graphically in figure 1. It will be seen that while the action potential is affected even at temperatures as low as 40°C., the curve becomes very steep at 42.5°C. and extinction under these conditions always occurred whenever the temperature was 43.2°C. or higher. Measured by the relatively gross method necessarily employed in these experiments little thermal shortening occurs below 45 to 46°C., although, in agreement with previous work (Schmitt and Wade, 1935) it was found that some shortening (1–2 per cent) occurs even at temperatures as low as 40 to 44°. Respiration and substrate activation appear to be unimpaired by 15 minutes' heating at temperatures up to 42° but are considerably affected at temperatures higher than 44°. At 43.2° which is the temperature of complete extinction of the action potential, neither respiration nor substrate activation is reduced more than about 10 per cent.

It is, of course, impossible to draw any definite conclusion from these data as to the immediate cause of thermal failure. It is conceivable that the very small shortening (1–2 per cent) or the small inhibition of energy

yielding processes (10 per cent) occurring at the extinction temperature may affect specific processes vital to the conducting mechanism. Until evidence can be adduced which would favor such a view, however, we have returned in our analysis to a consideration of the possible physical chemical

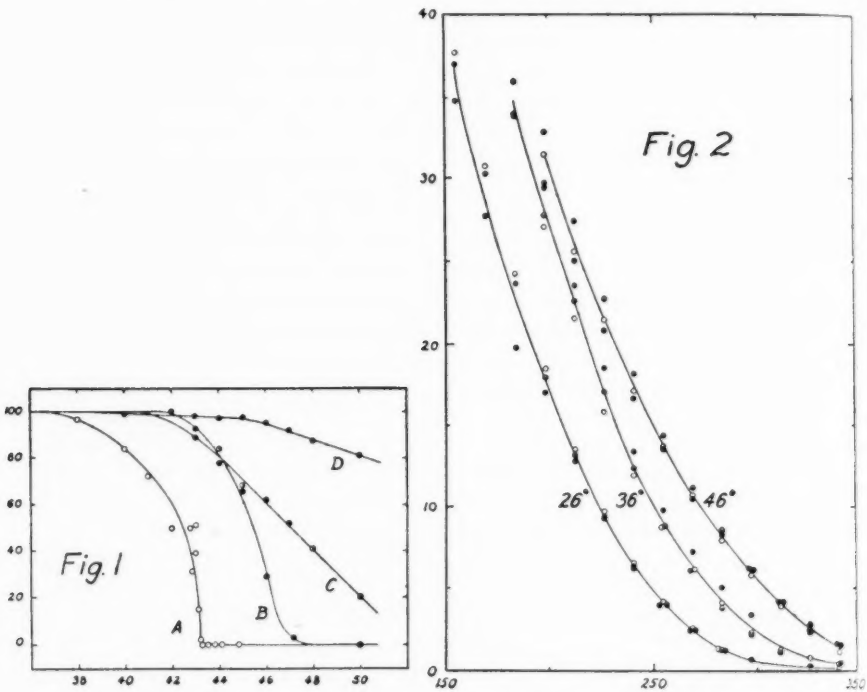

Fig. 1. The effect of temperature on nerve processes. Ordinates represent the particular nerve function referred to, in per cent of the normal (room temperature) value; abscissae represent temperature in degrees Centigrade. Curves represent: *A*, action potential spike height; *B*, oxygen consumption; *C*, substrate activation (methylene blue reduction); *D*, thermal shortening.

Fig. 2. Force-area curves of unimolecular films of a constant arbitrary amount of nerve lipoid spread on distilled water at three different temperatures. Ordinates represent force in dynes/centimeters; abscissae, area of the films in square centimeters. Open circles and half circles are data from independent films; closed circles are data on recompression of the half circle films.

or structural alterations which might be responsible for the effect. On the assumption that thermal failure may be due to a phase inversion of the lipoidal membrane, resulting in gross alterations of the electrical resistance, permeability, etc., of the membrane, we have studied the electrical con-

ductivity of poised emulsions of olive oil and of lecithin emulsions in Ringer solution. No abrupt change in conductivity was noted in any of these systems in the temperature range of 40 to 50°C. Pursuing a suggestion of Gasser (1931) that the effect of temperature on nerve processes may be due to a change in packing of a unimolecular film of lipoid situated at the critical interface, we have made a study of the behavior of monolayers of nerve lipoids spread on the surface of distilled water as a function of temperature. The results of these experiments are summarized in figure 2.

The only effect of temperature rise on these films is a continuous increase of area. The curve at 56° resembled that at 46° but was less reproducible due to melting of the ceresine with which the Adam-Langmuir trough was coated. The motion of talc particles shows that the films are fluid from 16° to 56°. They are not condensed, as they have no linear portion. Films spread on n/100 H_2SO_4 were entirely similar. Compressibility is high, resembling the films of pure sphingomyelin, phrenosin, and kerasin studied by Turner and Watson (1930) and the mixed oleic acid-cholesterol films of Adam and Jessop (1928). Lecithin alone and cephalin containing lecithin give curves of large compressibility; cholesterol alone is quite incompressible (Gorter and Grendel, 1928). Hence these films of total nerve lipoid are dominated by their unsaturated straight chain components. They do not collapse up to 40 dynes/cm., and on recompression of the same film give, within experimental error, the same curve. This variation, usually less than 3 dynes/cm., is comparable to that found by Harkins, Ries, and Carman (1935) for d-pimaric acid. Alcohol-ether extracts gave curves similar to benzene extracts. The failure to find an abrupt change in state of the film in the temperature zone at which nerve conduction fails, cannot be considered crucial with respect to the original suggestion of Gasser, however, since nothing is known of the rôle played by the hypothetical film or of the importance to the process of conduction of relatively small changes in the condition of the film.

Considerably more suggestive with regard to the rôle of lipoid orientation in thermal failure is the indication from x-ray diffraction that at or about the temperature at which the action potential is abolished, the radial orientation of the lipoid micelles is decreased (Schmitt, Bear and Clark, 1935). Upon the assumption that such a change in orientation should also manifest itself in a change in negative birefringence, a preliminary study was made of the birefringence of nerves heated for 15 minutes to 46°C. as compared with that of nerves at room temperature. These measurements revealed no significant difference in birefringence between the two groups.[2] This appears to be in line with the old observation that

[2] These measurements were made by Dr. R. S. Bear.

while nerve birefringence is decreased, even to extinction during heating, subsequent cooling causes the birefringence to be restored. The x-ray evidence indicates, however, that the micelles which give rise to the large spacing reflections, (171Å) do not become completely reoriented upon cooling, although individual lipoid molecules or small groups of molecules almost certainly do. The diffraction evidence of disorientation at the critical temperature, together with the optical evidence, will be discussed in more detail subsequently.

SUMMARY

By the adoption of uniform and reproducible conditions of heating, the cause of thermal inactivation of medullated nerve has been studied. Under these conditions the action potential is abolished at a temperature of about 43°C., at which temperature shortening amounts to only 2 per cent and respiration and substrate activation are reduced only about 10 per cent. A brief discussion is given of some of the physical and structural factors which may be more immediately involved in thermal failure.

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A BASIS FOR THE ANALYSIS OF VARIATIONS IN THE FORM OF ELECTROCARDIOGRAPHIC CURVES RESULTING FROM EXPERIMENTAL PREMATURE CONTRACTIONS[1]

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The work on the correlation of site of premature ventricular contractions with the contour of the resulting electrocardiographic curves has been given impetus by the recent reports on artificially induced premature contractions in the human heart (1, 2, 3). Unfortunately, since these results were not in accord with the previous studies of Lewis (4, 5) and also with the subsequent work of Katz and Ackerman (6), considerable difference of opinion has developed among the investigators as to the true value of the electrocardiographic data obtained.

It has, therefore, been thought worthwhile to reëxamine this subject in the hope that some common denominator might be found upon which to reconcile the apparent discrepancies in the previous results. The present report is based upon the systematic study of the electrocardiographic form of experimental ectopic beats obtained from numerous almost contiguous sites of stimulation on the epicardial surface of the cat's ventricles.

METHOD. The experiments were performed on thirteen cats under "dial" anesthesia, injected intraperitoneally. The chest was opened by removing the sternum, artificial respiration having been instituted previously. The pericardium was cut and used to make a hammock for the heart. Stimuli were then applied by means of two steel wires enclosed in a rubber base except for the tips, the latter being held lightly against the epicardial surface of the ventricles. When stimulating the posterior wall, an electrode with a 90° bend close to its tip was applied to the various desired spots; the normal position of the heart further being maintained by light pressure on its anterior surface with a gloved hand. A Harvard spring interruptor in the primary circuit of an inductorium was employed

[1] This study was aided by a grant from the American Association for the Advancement of Science.

to produce a series of ectopic ventricular contractions for each site of application, a rate slightly greater than that of the sinus pacemaker being used. The standard three leads were recorded in each instance. In a number of experiments, the heart was rotated to the right or left on its long axis, either by means of the gloved hand or by traction on ligatures inserted in its lateral walls, and the effect of this procedure on the electrocardiograms obtained from ectopic beats arising in definite areas on the ventricles was determined. In order to keep all other relationships of the heart unchanged during the rotation, constant manual traction in a caudal direction was maintained using a stay suture attached to the apex of the left ventricle.

RESULTS. *Influence of the point of stimulation on the electrocardiographic form of ectopic beats.* It was soon found that stimulation at random, even if limited to the same ventricle, resulted in different electrocardiographic curves in respect to form, direction and duration of the individual deflections in each lead. Accordingly, it was decided to investigate the matter systematically. Points at short distances apart were therefore stimulated, and in this step-like manner, the entire circumference of the heart was ultimately encompassed at various transverse levels. In each instance, stimuli were applied in the following sequence: the anterior, lateral and posterior surfaces of the right ventricle, the posterior aspect of the septum, the posterior, lateral and anterior surfaces of the left ventricle and finally the anterior aspect of the septum. Although leads I, II and III were obtained in succession for each site of stimulation, for expediency the presentation of data will deal only with the changes observed in leads I and III. The curves of lead II were found to conform either to those of lead I or more often to those of lead III.

When the records were examined in this manner, a clear cut correlation, characteristic for each lead, was found to exist between the site of stimulation and the resulting type of curve. A single experiment typical of all will be presented in detail. Figure 1 A demonstrates the type of curves recorded with lead I on stimulation of consecutive spots at a transverse level some distance below and parallel to the base of the ventricles. It will be noted that as the electrode is moved across the interventricular groove on either surface (fig. 2) a change in the contour of the curve takes place. The positive initial complex obtained with stimulation to the right of the groove gradually diminishes in amplitude to become almost isoelectric and then negative as the groove is passed and stimuli are applied to the left of it. A comparable but opposite type of change is observed in the terminal deflection.

For the same level and for the same sites of stimulation, entirely different results are recorded when using lead III (fig. 1 B). In this case, a transition in the type of curve is noticed some distance from the interventricular

sulci, on the lateral borders of the ventricles. The high positive initial complex obtained over the entire anterior surface of the heart at this level becomes smaller in amplitude, eventually to disappear as either lateral

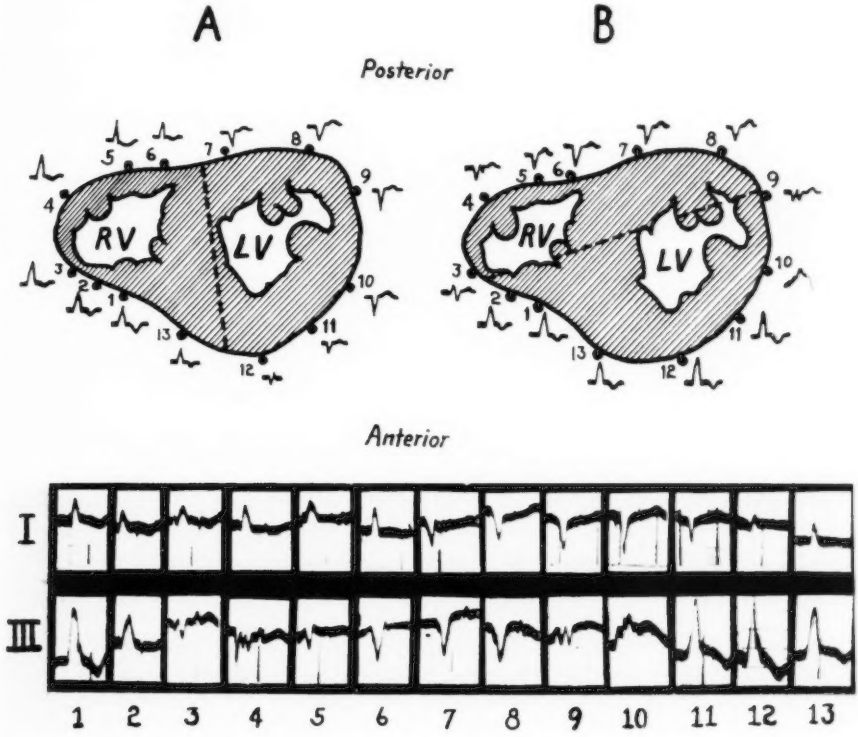

Fig. 1. Schematic drawings of a transverse horizontal section through the base of the ventricles, together with electrocardiograms obtained on stimulation of the various points designated. The numbers in the diagrams and those below the records correspond. A, diagrammatic representation of the type of curves obtained with lead I (copies of actual records below). B, diagrammatic representation of the type of curves obtained with lead III (copies of actual records below). It will be noted that no relationship as to direction and amplitude of the main wave exists between leads I and III. The broken lines in the drawings join the hypothetical lines of transition for leads I and III respectively. One centimeter = 1 millivolt. Time 0.2 second.

border is approached. It is replaced by a negative wave which becomes progressively larger as the posterior surface is reached, and remains large as the electrode is moved over the posterior aspects of both the right and

left ventricles. Again a comparable but opposite type of change is observed in the terminal deflection. Essentially the same results were

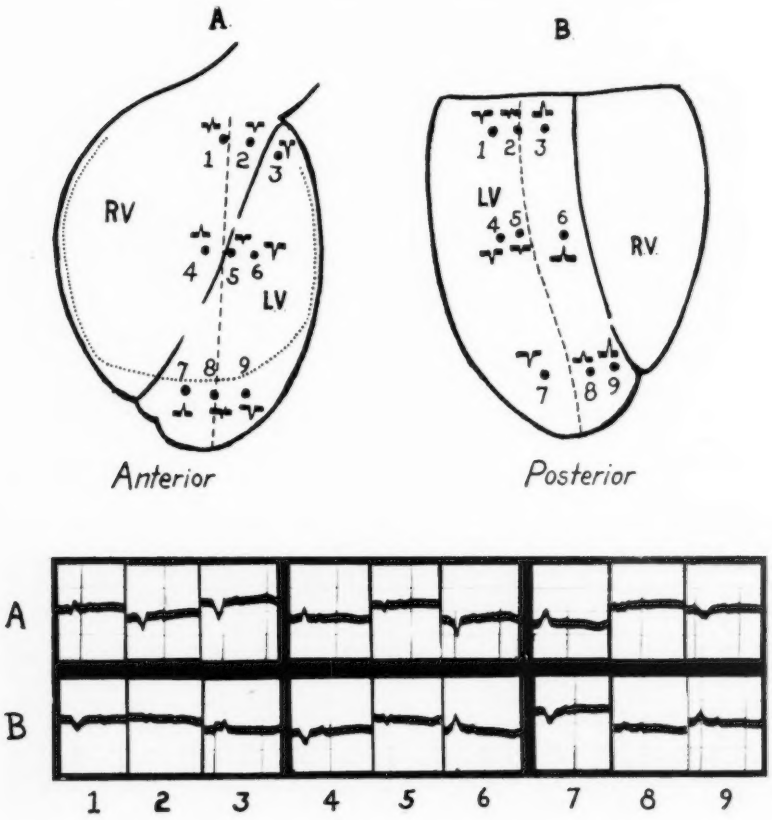

Fig. 2. Schematic drawings of anterior and posterior surfaces of the ventricles, together with electrocardiograms obtained on stimulation of the various points designated. All the electrocardiographic tracings were obtained with lead I. The numbers in the diagrams and those below the records correspond. A, tracings obtained on stimulation of the anterior surface of the ventricles. B, tracings obtained on stimulation of the posterior surface of the ventricles. The broken line in each drawing represents the hypothetical line of transition for the respective surface as recorded by lead I (see text). The dotted line in "A" represents the line of transition for lead III.

obtained for both leads I and III at other transverse levels, except in the vicinity of the inferior border of the ventricles (see below).

In other instances, the stimulating electrode was moved in the direction of the long axis of the heart from the auriculo-ventricular groove anteriorly, downward around the inferior border of the ventricles to the posterior surface and then upward to the base posteriorly. In lead I the type of curve generally remains unaltered by such a shift in the position of the electrode. In lead III, however, a definite transition is observed as the lower portion of the heart is approached. In the case of the right ventricle, the change takes place as the inferior border is reached, the major initial deflection, previously positive, reversing its direction when the posterior surface is stimulated. In the case of the interventricular septum and left ventricle the same type of reversal is observed, except that it usually manifests itself at a somewhat higher level, i.e., on the lower fourth of the anterior surface.

When the sites, which on stimulation give practically no initial deflection in lead I, are mapped out and connected, an hypothetical "line of transition" can be obtained. This line of transition for lead I arises anteriorly at the auriculo-ventricular groove, near the origin of the pulmonary artery, extends downward over the conus of the right ventricle, turns to the left to cross the anterior interventricular groove about midway between base and apex, and then passes obliquely for some distance onto the inferior portion of the left ventricle (fig. 2-A). It then curves around the apex of the heart to swing upward and somewhat to the left over the posterior surface of the left ventricle as far as the auriculo-ventricular groove posteriorly (fig. 2-B). A comparable line of transition can be obtained for lead III. It arises in the vicinity of the auriculo-ventricular groove on the right lateral border of the heart close to its anterior surface and extends inferiorly and somewhat anteriorly along this border to reach the anterior surface of the right ventricle a little above its extreme tip. From this point it turns to pass transversely to the left over the anterior inferior surface of the interventricular septum and left ventricle. It then curves upward and posteriorly to run along the left lateral border of the heart, reaching the auriculo-ventricular groove at the boundary of the posterior and lateral surfaces of the left ventricle[2] (fig. 2 A). It is realized that the lines of transition for leads I and III, thus obtained, may not coincide exactly in location with the actual ones in the intact animal, since on opening the chest and initiating artificial respiration, the position of the heart is changed somewhat, the apex in particular now pointing anteriorly (see below for the effect of change in the position of the heart).

Our results show, therefore, that the line of transition for lead I extends across both surfaces of the heart and does not strictly coincide with any anatomic boundaries separating the two ventricles. This line of transi-

[2] Preliminary experiments on four *rhesus* monkeys and three dogs reveal grossly the same location of the lines of transition for leads I and III.

tion, however, seems to have the tendency to divide the muscle mass of the outer walls of the heart into approximately two equal portions, a right and a left. Furthermore, both the anterior and posterior limbs of this line of transition are nearly at right angles to the recording line of lead I, i.e., a line extending between the two shoulders anteriorly. The line of transition for lead III likewise appears to separate the outer ventricular walls into two equal but entirely different masses. One portion, the anterior, is made up of the anterior outer wall of the right ventricle, the upper three-quarters of the interventricular septum and left ventricle and the left wall of the latter; the other, the posterior, includes the posterior

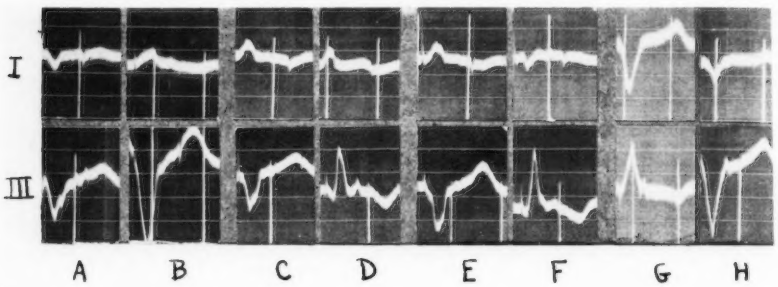

Fig. 3. Effect of rotation of the heart to the left so that the anterior surface is made up solely of right ventricle. Electrocardiograms recorded with leads I and III. A, records obtained on stimulation of the posterior surface of the left ventricle at the apex. B, after moderate rotation so as to bring this point of stimulation to the right of the hypothetical line of transition of lead I. C, records obtained on stimulation of the posterior surface of the right ventricle at the base. D, after moderate rotation so as to bring the point of stimulation anteriorly. E, records obtained on stimulation of posterior surface of right ventricle, near apex. F, after marked rotation so as to bring the point of stimulation anteriorly and to the left of the hypothetical line of transition for lead I. G, records obtained on stimulation of the anterior surface of the left ventricle at the base. H, after moderate rotation so as to bring the point of stimulation posteriorly.

wall of the right ventricle, the posterior wall of the interventricular septum and left ventricle as well as the anterior inferior one-quarter of the latter two.

It would be erroneous to localize, on the basis of the contour or direction of the electrocardiographic deflection, the site of origin of ventricular ectopic contractions, in the cat at least, to one or the other ventricle. Further, the horizontal level at which the impulse enters the conduction system does not appear to influence to any significant extent the direction of the initial deflection of these ectopic beats, except possibly in the case of lead III for sites of stimulation at the base and apex of the left ventricle

anteriorly. The initial deflection in lead I remains in the same phase so long as the stimulus is applied to the same side of the line of transition, regardless of whether the site of origin is near the apex or base. This is likewise true for lead III when the stimulus is applied to the posterior surface of both the right and left ventricles (fig. 3, C and E). Although it is realized that a vector derived from the main initial deflections of ectopic beats arising at the base of the ventricles differs from one arising near the apex, still the position of the point first excited relative to the cephalic and caudal aspects of the heart generally does not affect the *direction* of the resulting curve in any one lead.

Effects of rotation of the heart upon the location of the lines of transition. It can readily be appreciated that by rotating the heart to the right or left on its long axis, the relationship of any one portion of the muscle mass to the recording lead lines will be disturbed and consequently its electrical effects will be altered as well. It was therefore decided to investigate the effect of such a procedure upon the location of the lines of transition on the surface of the ventricles. Accordingly, in a number of experiments, these lines for leads I and III were obtained and related to anatomic landmarks on the surface of the ventricles. The lines were again determined after the heart had been rotated either to the right or to the left, and their position was found to have shifted. In the case of rotation to the left so that only the right ventricle presented anteriorly, it was found that the anterior portion of the line of transition for lead I extended entirely across this ventricle; stimulation of points to the left of it produced downwardly directed initial main deflections, (i.e., of a 'left ventricular type') even when the origin of the impulse was in the right ventricle (fig. 3, E and F). At the same time, the line of transition on the posterior surface was also displaced, but in an opposite direction, now extending across the left ventricle (fig. 3, A and B). A similar type of relationship was present on rotating the heart so that more of the left ventricle presented anteriorly, except that under these conditions the anterior part of the line of transition for lead I crossed only the left ventricle and the posterior part only the right ventricle. In other words, stimulation to the right of this line, either anteriorly or posteriorly, resulted in an initial deflection that was generally upward (a so-called right ventricular type) regardless of whether the site of stimulation was on the outer wall of the right or of the left ventricle. The line of transition for lead III, previously situated along the lateral borders of the heart, was displaced by the rotation either onto the anterior or posterior surfaces of the ventricles, the direction depending upon the type of rotation (fig. 3, C and D, G and H).

INTERPRETATION OF RESULTS. From an examination of the data obtained with the heart in the normal position and also following its rotation to the right or left, certain inferences can be drawn. It appears that the

type of curve obtained with leads I and III for ectopic ventricular beats depends generally upon the relationship of the site of stimulation to the line of transition for the lead. The further removed the stimulated part is from this line of transition, the more characteristic is the resulting curve; while when the stimulated point is in close proximity to the line of transition, bizarre and atypical records are obtained. The results so far as lead I is concerned can be correlated with the direction of the vector resulting from the spread of the ectopic impulse. Whenever the latter arises to the right and equidistant from the two limbs of the line of transition for lead I, regardless of what rotational position the heart is in, the excitation wave coursing through the heart will have an average direction to the left and parallel to the line of lead I. This would result in a large broad upright initial deflection in lead I. The reverse, as might be expected, is obtained whenever the impulse starts to the left and equidistant from the two limbs of the line of transition. In both instances the duration of the initial deflection is longer than normal because the excitation path is lengthened and consequently also the duration of the invasion period. Whenever the impulse starts at the line of transition, the excitation will spread equally to the right and left, and as might be expected, the resulting record consists of small polyphasic initial deflections of short duration (an observation previously made by Rothberger and Winterberg (7)). Between these extremes intermediate curves are obtained. Thus, as far as lead I is concerned, the type of curve obtained with artificial stimulation does not depend upon which ventricle is first activated (although grossly and merely as fortuitous finding this appears to be true when the heart is in its normal position) but upon the relationship of the point of origin to the line of transition. This line of transition appears to divide the muscle mass composing the walls of the two ventricles and septum equally and approximately at right angles to the recording line of this lead. Furthermore, with stimulation of a definite spot, the distortion in the deflection of the ectopic beats consequent to rotation can also be readily understood by comparing the altered path of excitation through the mass of heart muscle with the fixed line and direction of the recording lead. Examining the data from a somewhat different angle, it was found that the line of transition obtained after rotation usually bore practically the same relationship to the recording lead line as the one obtained with the heart in its normal position; and further that it remained in almost an identical position in respect to the body as a whole, even though its location on the heart surface was changed in proportion to the degree of the rotation. However, on some occasions, the position of the line of transition in reference to the lead line did shift slightly when the heart was rotated. This might be explained on the fact that the heart can not be compared exactly to a muscle shell

of equal thickness (in view of the differences in thickness of the outer walls of the right and left ventricles), and as a result of the rotation, the relationship of muscle mass to lead line might have been altered, so that a consequent change in direction of the line of transition occurred.

A similar relationship was demonstrated to exist between the location of the site of impulse-formation and the line of transition of lead III, on the one hand, and the direction magnitude and duration of the initial deflection on the other. However, this could not be explained on a simple vector analysis such as was found to hold in lead I. Either the correlation with vector analysis in lead I is as fortuitous a relationship as the location of its line of transition in the vicinity of the interventricular grooves, or some other factors are present in the case of lead III which modify the effect anticipated from the vector concept.

SUMMARY

Analysis of electrocardiographic curves obtained by systematic application of stimuli to the epicardial surface of the cat's ventricles revealed the existence of lines of transition for leads I and III. These lines of transition appeared to divide the ventricles into two equal, but in each case different, muscle masses, without strict regard to any anatomic boundaries. The line of transition for lead I ran almost vertically downward over the anterior surface of the heart and then curved around the apex to extend upward over the posterior surface, being situated on the left ventricle, near its right border in most of its course. The line of transition for lead III extended along the lateral borders of the heart except inferiorly where it curved over the anterior surface of the left ventricle and interventricular septum.

Rotation of the heart on its long axis did not materially affect the location of these lines of transition with respect to the body. Their position on the heart was changed in proportion to the degree and direction of rotation. These results provide further evidence in support of the view that the duration and contour of the initial deflection of ventricular ectopic beats depend upon the location of the impulse initiation with reference to the hypothetical line of transition for a specific lead and so upon the general direction of the impulse spread in relation to the recording line of that lead. These results further support the view that the contour and duration of the initial complex obtained by stimulation of ventricular sites do not depend wholly upon whether the Purkinje system of one ventricle is excited in advance of that of the other. The level at which the stimulus is applied in reference to the long axis of the body plays but a negligible part in determining the type of complex produced.

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STUDIES ON THE LEVEL OF ANESTHESIA FOR THE OLFACTORY AND TRIGEMINAL RESPIRATORY REFLEXES IN DOGS AND RABBITS

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This problem is an outgrowth of some inhalation tests made on dogs that were to be used for conditioned smell reflexes, which disclosed that moderate barbital hypnosis abolished all respiratory inhibition from olfactory stimulation.[1] It finally includes the effects of numerous olfactory and trigeminal stimulations on respiration from at least six dogs and an equal number of rabbits for each anesthetic used.

In some preliminary experiments with dogs (technique described in previous paper) it was demonstrated that lavender, anise, cloves and asafetida[2] inhibited respiration only over the olfactory nerve, while chloroform, eucalyptus, pyridin (close up) and sheep dip acted over the trigeminal system as well as the olfactory, but not over the vagus in the time alloted for inhalations. Lavender proved the most effective of the olfactory stimulants.

PROCEDURE. The following anesthetics were used—ether, morphine, sodium amytal (Lilly)[3] dial (Ciba), nembutal (Abbot) and barbital sodium. All injections were intraperitoneal unless otherwise stated. Both small and large doses of anesthetics were used and the tests were made during induction and during recovery. Thoracic respiration was recorded on a kymograph by a tambour connected to a balloon strapped to the chest wall or in small rabbits by a lever pinned to the skin of the chest. The animal was either blindfolded or blinded and ears were plugged with cotton. Inhalation was from wide-mouthed bottles containing cotton and a few drops of the inhalant. Very volatile vapors like chloroform were held about 20 mm. from the nostrils, while the less volatile odors lavender, anise, etc., were held about 10 mm. distant. Empty bottles

[1] The writer had previously reported for rabbits that the respiratory and pulse inhibition from inhalation of the purely olfactory vapors orange, lavender and cloves were fully as pronounced under deep hypnosis as under no anesthesia.

[2] Asafetida and other disgusting odors were not used in rabbits for they have been shown to have no effect on respiration, blood pressure or pulse in this animal.

[3] The sodium amytal used in these experiments was generously donated by the Eli Lilly Co.

served as controls. Before making a test during the first stage of anesthesia the animal was always awakened if sleeping.

DOG EXPERIMENTS. *Barbiturates.* A typical test is selected from an 8 kilo dog given 5.5 cc. nembutal at 1:55 p.m. From this time on the results of some of the many inhalation and other reflexes are tabulated, using the following symbols: +, signifies normal response: 0, no response; I, considerable inhibition or sluggish (as used for respiratory reflex—distinctly depressed and slowed); SI, slight inhibition.

TIME	CHLOROFORM RESPONSE	LAVENDER RESPONSE	CLOVE RESPONSE	ANISE RESPONSE	ASAFETIDA RESPONSE	PAIN (PINCHING NOSE)	PAIN (PINCHING FOOT)	SWALLOWING REFLEX	CORNEAL REFLEX	PUPILLARY REFLEX	TENDON REFLEX (K. K.)	INVOLUNTARY MOVEMENTS	RIGHTING REFLEX	SOUND (Shrill whistle)	SOUND (Speaking)	SIGHT REFEX (MOVING OBJECTS)	VOLUNTARY MOVEMENTS	STAGE OF ANESTHESIA
2:25–2:50	0	0	0	0	0	0	0	0	0	0 SI?	I	0	0	0		0	0	3d.
4:15–4:20	+	0	0	0	0	+	0	I	0	SI	+	0	0	0		0		
5:18–5:25	+	0		0		+	+	+	+	+	+	+	0	0		0		2d.
6:20–6:25	+	0	0	0								+		+?		0	0	
6:40		0			0									+		0	0	
7:10–7:15		0	0	0	0							+		+				1 st.?
7:30													+	+	+	0	0	
8:02																+	+	
8:30		0																
8:40–8:50		0+	0	0	0													
9		+	0	0														
9:10–9:15		+	+	+	+													

Figure 1 demonstrates: A, the trigeminal-respiratory reflex which occurred from inhalation of chloroform at 4:15; B, the absence of the olfactory-respiratory response at 8:30 from lavender; C, the first response from lavender at 8:46 and D, the first response from anise at 9:15. At 9:16 the dog walked about the room, falling but once. The foregoing data and tracings were corroborated by other dogs under nembutal and other tests under sodium amytal, dial and barbital sodium.

Ether. The dog selected to illustrate the stage of ether which would abolish the olfactory-respiratory reflex was given ether until he walked with an unsteady gait. At this time inhalation of lavender and cloves resulted in considerable depression and slowing of respiration accompanied by sniffing and turning of the head. The dog was carried still deeper into the first stage of anesthesia until unable to remain on his feet and response to sound was good. At this stage lavender and cloves produced no effect

on respiration, but the trigeminal-respiratory response from chloroform was strong. The animal was finally carried well into surgical anesthesia and no further ether was given. At this time (3:55 to 3:57) when there were no corneal, swallowing, pain (pinching foot) reflexes, but a response from pinching the nose, the irritant chloroform (fig. 1 M) elicited a slight depression of respiration. At 4:09 there was no response to sound

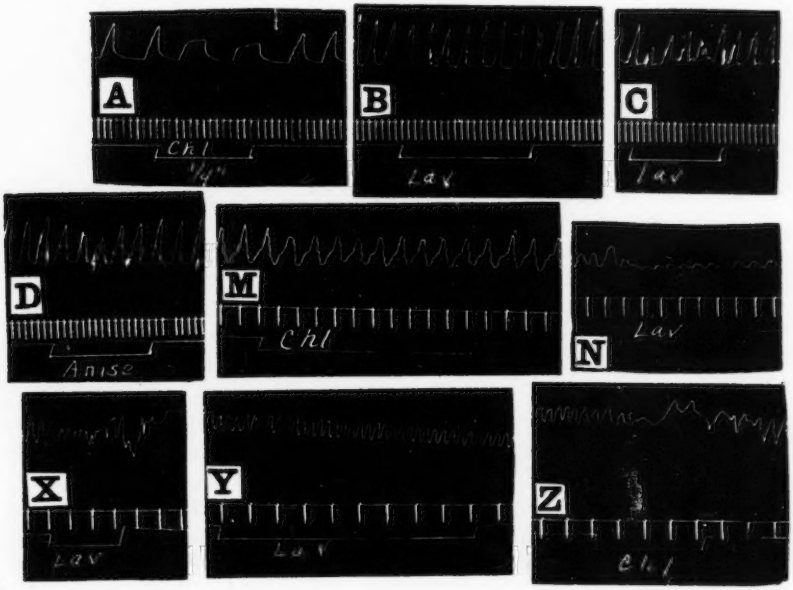

Fig. 1. Dog records—top row a thoracic respiratory graph, middle row time in seconds and bottom row interval of inhalation. A is a positive chloroform response during late third stage of nembutal anesthesia; B, a negative lavender response during first stage of nembutal; C and D, first responses to lavender and anise earlier in first stage than B. M is a chloroform response during late third stage of ether and N first lavender response in first stage. X shows lavender response during light morphine; Y and Z, a negative lavender and a positive chloroform during deeper morphine.

(whistle) or inhalation of lavender, but the sight reflex to moving objects was present. At 4:10 the sound reflex returned, but the first olfactory-response to lavender (fig. 1 N) did not appear until a minute later. At 4:13 lavender evoked a pronounced depression and slowing of respiration and cloves produced its first effect on respiration. After this the dog was able to walk but with an unsteady gait.

Morphine. The following experiment is illustrative of the effects of

the early stages of morphine narcosis on respiratory reflexes from olfactory and trigeminal stimulation. A 7.2 kilo dog was given 10 mgm. (1.3 mgm. per kgm. weight) morphine sulfate subcutaneously. At various intervals after injection the negative or positive effect of the four olfactory stimulants and one trigeminal-olfactory stimulant (chloroform) is tabulated.

TIME AFTER MORPHINE	INHALATION ASAFETIDA	INHALATION ANISE	INHALATION CLOVES	INHALATION LAVENDER	INHALATION CHLOROFORM
minutes					
5–8	+	+	+	+	+
20–30	+ and 0	+	+	+	+
32–35	0	0	0	+	+
45–50	0	0	0	0	+

Immediately after recording the last tracing in which lavender, the strongest olfactory stimulant used, failed to depress respiration or cause any movements, and when the mild irritant chloroform gave a good depression of respiration, the following reflexes were normal or practically normal: sound (calling by name), sight (moving objects), righting, pain, (pinching foot), swallowing, tendon (k.k.) and corneal. At this stage respiration was fast and pupil was considerably constricted but responded fairly well to a flash of light. During the entire experiment the dog was able to stand, walked with a wide base and was slightly unsteady on stairway. In figure 1, tracing X shows the lavender response 34 minutes after morphine; tracing Y the absence of the lavender response 45 minutes after morphine; while Z reveals a conspicuous respiratory response to chloroform 50 minutes after morphine.

Similar tests taken during the recovery from morphine gave like results as did other tests with increased dosage.

RABBIT EXPERIMENTS. *Barbiturates.* A 2 kilo rabbit given 1.5 cc. of nembutal intravenously at 8:55 is selected for comparison with the previously described dog. Between 9:15 and 9:55 when rabbit was under surgical anesthesia (no swallowing, corneal, pupillary or pinching-of-foot reflexes) inhalation of the olfactory vapors, lavender (fig. 2 A), cloves and anise evoked no change in respiration, while chloroform (fig. 2 B) depressed and slowed respiration as an irritant. The first response to lavender (fig. 2 C) occurred at 9:56. From 10 to 10:10 no respiratory response could be obtained from anise or cloves, but from 10:10 to 10:16 and on, anise and cloves depressed and slowed respiration consistently and the response to lavender was much more pronounced than shown in figure 2 C. From 10:20 to 10:25 the corneal, pupillary, swallowing, pain (pinching foot) reflexes were sluggish. At 10:45 these reflexes were good but no sound or sight reflexes were present. At 11:20 rabbit raised head from floor for first time, saw approaching objects, responded to sound?, fell

when placed on feet and slept when undisturbed. First voluntary movements appeared at 11:35. At 12:08 stood up and jumped around for first time, but forefeet slipped outward when jumping. This is about the stage of recovery in dogs when the olfactory-respiratory reflex first appears.

The inhalation tests for sodium amytal, dial and barbital sodium gave results very similar to those for nembutal. In every instance the trigeminal-respiratory reflex from inhalation of chloroform persisted well into the third stage of anesthesia, being abolished about the same time as

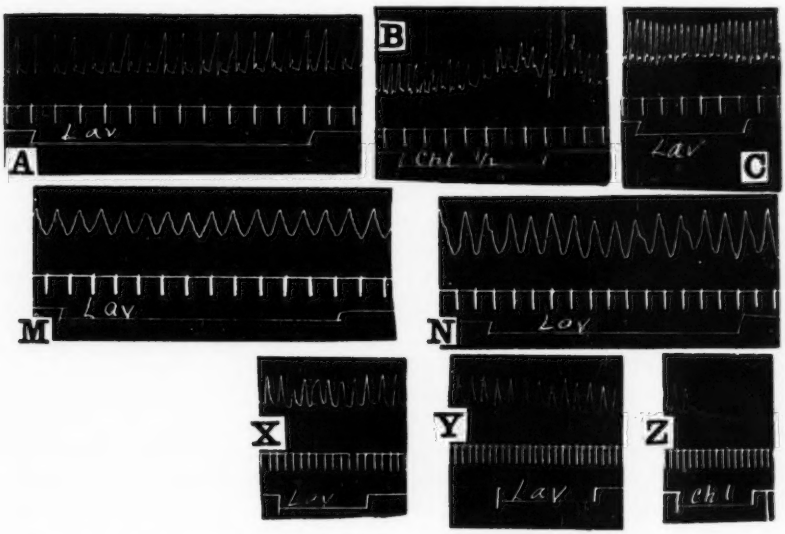

Fig. 2. Rabbit records—A and B are negative lavender and positive chloroform responses during surgical anesthesia from nembutal; C, first lavender response during lighter surgical state than A and B; M, no response from lavender under deep surgical ether and N first lavender response during lighter surgical state than M; X slight response to lavender under deep morphine and Y and Z, no response to lavender and good response to chloroform under still deeper morphine.

the pain reflex from pinching the nostrils. The olfactory-respiratory reflex, however, was not extinguished until early in the third (possibly late second in some instances) stage.

Ether. The experiment selected for the effects of ether on the olfactory-respiratory reflex was from a 4.5 kilo rabbit carried deep into the third stage of anesthesia, during which the swallowing, corneal, pupillary and pain (pinching foot) reflexes had disappeared and only very slight movement resulted from pinching the nostril. At this stage chloroform elicited some slowing and depression of respiration, while the purely olfactory

stimulants, lavender (fig. 2 M), cloves and anise in no way altered respiration. Two minutes after stopping ether, when the above mentioned reflexes were absent or practically so, but a strong response resulted from pinching the nose, lavender (fig. 2 N), anise and cloves caused some depression of respiration. The effect of cloves and anise was fully as pronounced as lavender. The first appearance of the olfactory-respiratory reflex was obviously in the third stage of anesthesia, shortly after the trigeminal but a long time before sight and sound or any body movements.

Morphine. It should be noted that the amount of morphine required to abolish the olfactory-respiratory reflex varied considerably. Some rabbits required double the dosage of others.

As illustrative of a typical experiment, a 2 kilo rabbit given 112 mgm. of morphine sulfate in several instalments finally reached a stage of narcosis during which only a very minute respiratory response (fig. 2 X) could be obtained from inhalation of lavender. At this stage of narcosis the pupillary, pain (pinching foot) and swallowing reflexes were considerably diminished, no definite response resulted from sound or moving objects, the animal lay with legs outstretched, limp and motionless. Eight minutes later lavender (fig. 2 Y) evoked no respiratory response, while chloroform (fig. 2 Z) arrested respiration completely through trigeminal stimulation. During this stage of slightly deeper narcosis the pupillary, swallowing and pain (pinching foot) reflexes were either sluggish or absent, the corneal reflex was fair, while the response from pinching or even touching the nostrils was good.

After making due allowance for the distinctive characteristics of morphine it will be seen that its action on the olfactory-respiratory reflex is comparable to that of ether and the barbiturates. The extinction of this reflex in rabbits does not occur in light narcosis as in dogs but in much deeper narcosis, disappearing a little before the trigeminal-respiratory reflex.

Chloroform as an olfactory stimulant. The following tests were made to make certain that the more delayed response in the previous experiments from chloroform is due to its trigeminal action and not to its being a stronger olfactory stimulant. Since the results in dogs or rabbits were identical for the same stages of anesthesia from nembutal, ether and morphine, the illustrative tests for dogs happened to be with morphine and those for rabbits with ether.

A 7 kilo dog in which the afferent impulses from chloroform stimulation were limited to the olfactory nerves[4] was given 16 mgm. of morphine sulfate subcutaneously at 11:33 a.m. A uniform light narcosis occurred between 11:50 and 12:15 during which the corneal, tendon, pain (pinching

[4] Technique described in detail in previous paper.

foot), righting and sound reflexes were normal and the animal was able to walk with little or no unsteadiness. Inhalation of lavender at 12:14 and chloroform 15 seconds later elicited about the same depression of respiration (fig. 3 A and B). A movement of the chloroform bottle to within 5 mm. of the nostril produced the additional depression shown in the latter part of tracing B. At 12:15 the dog was given 8 mgm. additional morphine. From 12:22 to 12:23 chloroform (fig. 3 C) and lavender (fig. 3 D) evoked no respiratory changes. Following this the above mentioned reflexes were found to be normal and the dog walked with but little wabbling.

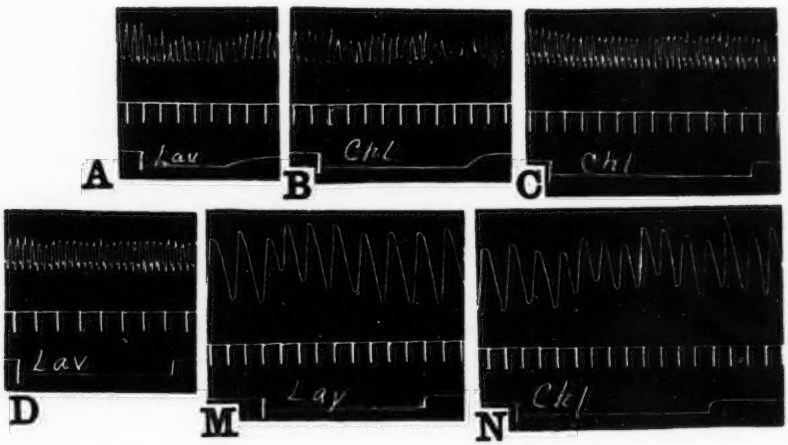

Fig. 3. Animals in which afferent impulses were limited to olfactory nerves—A and B, responses from lavender and chloroform from dog under very light morphine; C and D, no response in dog from chloroform and lavender during slightly deeper morphine. M and N, first and very similar responses from lavender and chloroform from a rabbit during early third stage of ether.

A 2 kilo rabbit in which the inhalation impulses affecting respiration were limited to the olfactory nerves was carried deep enough into the third stage of ether anesthesia to have abolished the respiratory reflex from inhalation of chloroform in a trigeminal-intact animal. Ether was stopped and several negative lavender, chloroform and anise tracings were obtained. An interval of several minutes elapsed before the first positive respiratory response came from anise. This was followed shortly by cloves, chloroform (fig. 3 N) and lavender (fig. 3 M) in the order mentioned. It is apparent from the tracings that chloroform causes no more depression of respiration than lavender at this stage of anesthesia. Later tracings disclosed the greatest depression coming from anise.

DISCUSSION. The first effect of ether suggests cortical depression. Morphine also depresses the sensory cortex early, but it first produces vomiting and respiratory changes in dogs. Maloney and Tatum have shown that decerebration in no way altered the respiratory depression from morphine and one would expect the vomiting reflex to occur after decerebration. Amsler has demonstrated that an amount of morphine sufficient to delete pain symptoms in rabbits failed to depress these symptoms after decortication.

Investigations on the barbiturates by Pick, Silver, Keeser and Keeser, Koppanyi et al., Forbes et al., Fulton and Keller, Pavlov, Wolff and Gantt, Page and Coryllos, Pearcy and Weaver and Fulton and Wolff's discussions of Bucy's paper reveal one group favoring diencephalic action of this hypnotic and another cerebral action. The much quoted interpretation of the findings (not corroborated by Koppanyi et al.) of Keeser and Keeser that the presence of barbital in the thalamus and corpus striatum and absence in other areas of the brain after injection signifies that the site of action of barbital is limited to these two areas, causes one to wonder if the opposite deduction is not equally tenable, namely, that its absence in cerebral cortex indicates an earlier consumption, its site of action, which in turn fits into Pavlov's theory for sleep.

The exact place of action, the intensity and rate of activity of any anesthetic has been determined for very few if for any reflexes. In conditioned reflexes the site of action may be in the afferent synapse in the thalamus, in the sensory, motor or association synapse in the cortex or in a lower synapse in the motor thalamus or reticular formation. In an olfactory conditioned reflex it might occur in a primary afferent center (bulb, perforated space, amygdaloid nucleus or paraolfactory area) in any part of the general cortex, dentate gyrus and hippocampus, or in many places in the thalamus or formatio reticularis. It may occur in three places in a medulla reflex. Possibly the formatio reticularis is more important than is generally considered. It seems very probable that the anesthetic block for any area might differ considerably for two very different species of mammals, though the two reflexes sight and sound seemed to disappear at about the same stage of anesthesia in rabbits as in dogs.

With so little data of this type available on anesthetics one can at best only speculate as to why the olfactory-respiratory reflex disappears so early in anesthesia in dogs and so late in rabbits. Three tentative hypotheses suggest themselves: 1, that this reflex in dogs is more closely associated with the cerebral cortex than in rabbits; 2, that only the brain stem was involved in either case, but that the rabbit possessed more direct fiber connections with the spinal cord; 3, that the thalamic relays were chiefly utilized in either case, but the sensitivity of the thalamus for anesthetics differs considerably in these two animals.

On the other hand one would expect the blocking of the trigeminal-respiratory reflex by anesthetics to take place in the medulla, for this reflex occurs in "midbrain animals" and is abolished at the same level of anesthesia in dogs as in rabbits and the stage of its extinction is always deeper than the level for the olfactory-respiratory reflex.

SUMMARY

The olfactory-respiratory reflex from inhalation of lavender, anise, and cloves was abolished during the first stage of anesthesia from ether, morphine and four barbiturates in dogs, while in rabbits it was not extinguished until the early part of the third stage.

The obliteration of the trigeminal-respiratory reflex from inhalation of chloroform occurred about the middle of the third stage of anesthesia in dogs and rabbits.

When stimulation from chloroform inhalation was limited to olfactory conduction the respiratory reflex was abolished fully as early by ether, morphine and nembutal as it was from the purely olfactory stimulants.

In view of our limited knowledge of the relative intensity or speed of action of any anesthetic to any encephalic area it is unwise to attempt an explanation for the discrepancy in time of extinction of the same or different reflexes in dissimilar species of animals.

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LACTATION IN ADRENALECTOMIZED RATS

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Several recent reports have indicated that the adrenal cortex may be a factor of importance in the physiology of lactation. Carr (1931) observed that a cortical extract, which he made after the Swingle-Pfiffner method, would maintain life but would not support lactation in adrenalectomized rats. One of us (Gaunt, 1933) reported that adrenalectomized rats could deliver normal litters of young but generally would not lactate sufficiently to raise them unless large accessory adrenals were present. We have since observed this same phenomenon in several other cases, including a series prepared as controls for this study. Brownell, Lockwood and Hartman (1933) offered evidence indicating that there was a hormone in the adrenal cortex designated *cortilactin*, distinct from the life-maintaining hormone, which was essential for lactation. Swingle and Pfiffner (1932) found that their cortical extract would sustain normal lactation in adrenalectomized dogs. A species difference, extract difference, or some other source of discrepancy is indicated. Britton and Kline (1935) reported serious lactation deficiencies in cats and rats after partial adrenalectomy. This report concerns a further study of the rôle of the adrenal cortex in lactation; and includes some observations concerning the hypothesis that there is a separate lactogenic hormone of adrenal origin.

METHODS. The adrenals were removed from young, healthy, pregnant females, one to five days prior to delivery, and treatment with the various preparations begun at the time of operation. Operative effects did not influence results (see series A-V). Daily weight records were kept of the young as a rough quantitative index of the amount of milk secreted by the mothers. The litters began to eat at about 20 days age, after which growth is not entirely dependent upon the mother's lactation. The litters were reduced to four in number at the time of birth. This made all comparative records uniform, and gave a better indication than would larger litters of whether any lactation at all was occurring. The same diet, type

[1] Part of this work was done at The Biological Laboratory, Cold Spring Harbor, through the courtesies of the late Dr. R. G. Harris.

of operation, and care used by us in other adrenal studies were used here (Gaunt, 1933; Gaunt, Tobin and Gaunt, 1935).

The cortical extracts used were supplied largely by Dr. W. W. Swingle of Princeton University, and in part by Dr. Oliver Kamm of Parke, Davis & Co. The Follutein and Prolactin were furnished by Dr. J. A. Morrell of E. R. Squibb and Sons. For these materials the authors are greatly indebted.

Normal lactation in our colony. No instance of lactation insufficient to raise the young has ever occurred among our normal animals.[2] Failure to care for and suckle the young has never occurred except in a few cases where the precaution was not taken to remove the mothers from stock cages before delivery. The average growth rate during the suckling period has been kept on 43 typical normal litters, averaging 7.7 pups each. The time of weaning was from 28 to 30 days, and at 30 days the average weight was 56.3 grams ± 1.46 (the standard error). The fact that our experimental litters were reduced to four, while the normals averaged 7.7 in size, exaggerates the growth differences between them; but the more significant comparisons here are those between the variously treated experimental litters, all of uniform size.

Extracts used: Explanation of "series" groups. These experiments were begun late in 1933. The cortical extracts used in the first experiments were of about equal potency. They kept all the lactating adrenalectomized animals alive during treatment without exception. Experiments with these extracts are listed as "series A." Our final set of experiments was done with extract furnished by Doctor Swingle in 1935. This material was at least three times as potent in its life-maintaining properties as that previously used. The doses were reduced accordingly. Experiments with this more potent extract are listed as "series B."

Series A-I. Lactation in adrenalectomized, cortical extract-treated females. Ten pregnant females were adrenalectomized and put on 1 cc. per day of cortical extract (divided doses, twice daily). The results are shown in table 1. It is seen that in only four cases were the litters raised. In all cases the young were malnourished, and showed a sub-normal growth rate. Parturition was apparently normal and without difficulty. Usual maternal care was attempted for all but one litter. In every case at least a little milk was secreted after delivery and could be seen in the stomachs of the young through their body walls.

The presence or absence of accessory adrenals did not influence lactation in these animals. The same was true in subsequent experiments. Five mothers (see table 1) lived indefinitely after treatment was stopped

[2] An occasional death among the normal suckling young was observed in approximately one per cent of the cases. That this was not due to lactation deficiencies in the mother was indicated by the growth of the litter mates.

and showed accessories when killed for autopsy some months later. Of this group two raised their litters and three did not. The other five animals lacked functional adrenal accessories—at least they died after treatment was stopped. In these five cases, likewise, two mothers raised their litters and three did not. Accessories when present were probably of no benefit because with the operations done shortly before delivery, hypertrophy was not sufficiently rapid to permit a significant amount of cortical secretion.

TABLE 1

Series A-I: Given 1 cc. cortical extract per day
(Litters of 10 treated mothers: 4 lived, 6 died)

LITTER NUMBER	AVERAGE WEIGHT OF SUCKLING YOUNG				CHANGE IN WEIGHT OF MOTHERS WHILE TREATED	NUMBER OF DAYS TREATED	LIFE-SPAN OF MOTHERS WHEN TREATMENT STOPPED
	On 1st day	On 10th day	On 20th day	On 30th day			
	grams	*grams*	*grams*	*grams*	*per cent*		
300*	4.7	10.2	12.0	20.0	−9.8	26	Lived indef.
307*	5.5	13.5	16.2	31.0	−14.0	20	Lived indef.
311*	4.5	12.2	16.2	25.0			60 days
336				21.0			15 days
301*			Died on 8th day		−1.0	10	Lived indef.
303*		9.5	Died on 20th day		−1.0	21	30 days
332			Died on 2nd day				8 days
337			Died on 3rd day				Lived indef.
338			Died on 3rd day				Lived indef.
340			Died on 4th day				2 days
Average of series A-I	4.9	11.4	14.6	24.2			
Average of normal litters	5.0	14.5	26.0	56.3			

* Given Parke-Davis Co.'s *Eschatin*. Cortical extract for all other animals here and in subsequent experiments supplied by Dr. W. W. Swingle.

The above experiments would seem to confirm the theory that something essential for lactation is lacking in the Swingle-Pfiffner life-maintaining extracts of the adrenal cortex. That such an interpretation is misleading is subsequently shown.

Series A-II. Lactation in adrenalectomized, salt-treated females. It is known that treatment with salt solutions will palliate or prevent the occurrence of adrenal insufficiency in various species of adrenalectomized animals including the rat. (References given in Gaunt, Tobin and Gaunt, 1935.) The effect of these solutions in sustaining lactation was studied.

Nine females, adrenalectomized before delivery, were given either normal saline or the Rubin-Krick (1933) solution to drink. The results of this treatment are shown in table 2, and are similar to those obtained with 1 cc. cortical extract. Three litters lived and six died. Treatment was continued for 30 days even if the litters died before that time.

The success of lactation in these cases seemed to depend largely on the amount of salt solutions consumed by the mothers. The average amount of fluid consumed per day by the mothers which raised their litters was 62.5 cc.; while the figure for those unable to raise their litters, during the

TABLE 2

Series A-II: Salt treated

(Litters of 9 treated mothers: 3 lived, 6 died)

LITTER NUMBER	AVERAGE WEIGHT OF SUCKLING YOUNG				CHANGE IN WEIGHT OF MOTHERS DURING TREATMENT	NUMBER OF DAYS TREATED	LIFE-SPAN OF MOTHERS WHEN TREATMENT STOPPED
	On 1st day	On 10th day	On 20th day	On 30th day			
	grams	*grams*	*grams*	*grams*	*per cent*		
362	4.5	9.0	17.5	40.0	+9.7	30	8 days
364	5.0	11.7	18.5	35.0	−8.0	24—day of death	
368	6.0	15.2	24.0	50.0	+26.0	30	Lived indef.
363	5.0	11.0	Died on 14th day		−19.0	17—day of death	
365			Died on 3rd day			23—day of death	
366			Died on 4th day		−1.0	30	Lived indef.
367	4.5	7.0	Died on 13th day		+9.6	30	Lived indef.
370	5.0	12.6	Died on 18th day		+5.7	30	9 days
371			Died on 2nd day		+8.7	30	Lived indef.
Average of series A-II	5.0	11.3	20.0	41.7			
Average of normal litters	5.0	14.5	26.0	56.3			

period that their young were still alive, was 33.4 cc. per day. Animal 362 was a noteworthy case. Her abbreviated protocol follows:

Animal 362 was adrenalectomized 3 days before delivery. Weighed 211 grams immediately after delivery, dropping within the next four days to 194.5 grams. She then began drinking enormous amounts of normal saline, oftentimes over 100 cc. per day, and on one day 160 cc. While lactating in approximately normal amounts she gained to a peak weight of 255.5 grams. She appeared in the best of health, although so filled with fluid that she looked bloated. When the salt solution was

discontinued and distilled water given her to drink, she dropped within 8 days to 160 grams weight (a 37 per cent loss from peak weight) and died. The growth of her litter is indicated in table 2.

Series A-III. Lactation in adrenalectomized rats treated with cortical extract and salt. As seen above neither the dosages of cortical extract given nor salt treatment alone supported a normal lactation. We found that these two treatments could be combined with more success.

Seven pregnant females were adrenalectomized, given normal saline to drink, fed a diet to which 2.5 per cent NaCl was added, and given 1 cc.

TABLE 3

Series A-III: Treated with salt and cortical extract

(Litters of 7 treated mothers: 6 lived, 1 died)

LITTER NUMBER	AVERAGE WEIGHT OF SUCKLING YOUNG				CHANGE IN WEIGHT OF MOTHERS DURING TREATMENT	NUMBER OF DAYS TREATED	LIFE-SPAN† OF MOTHERS WHEN TREATMENT STOPPED
	On first day	On 10th day	On 20th day	On 30th day			
	grams	*grams*	*grams*	*grams*	*per cent*		
440	5.0	14.5	26.0	48.0	+21.8	27	10 days
441	5.2	11.7	17.0	42.2	+10.2	29	20 days
442	5.2	11.7	16.2	35.2	+10.0	29	8 days
445	4.7	10.7	16.7	39.0	+14.6	26	20 days
384	4.2	15.0	29.5	60.0	+24.0	26	11 days
570	5.0	12.5	18.0	38.0	+4.8	22	7 days
444	5.2	Died at 7 days*					
Average of series A-III	5.0	11.7	20.6	43.7			
Average of normal litters	5.0	14.5	26.0	56.3			

* Mother deserted litter; was lactating adequately at the time.

† Dated from time of withdrawal of salt; extract discontinued 5 days earlier.

per day of cortical extract (divided doses, twice daily). The results are shown in table 3. The growth rate of six of the seven litters was either close to or within the range of normal variation. One litter died as a result of the mother's desertion rather than insufficient lactation.

The mothers on extract and salt treatment, during the first 20 days of their lactation period, drank an average of 41.2 cc. of salt solution per day, compared to the consumption of 62.5 cc. by those lactating on salt solutions alone. All mothers gained weight while lactating, but lost weight rapidly when treatment was discontinued.

It is well established that after adrenalectomy there is a severe disturbance in fluid and salt balance (Swingle, Pfiffner, Vars and Parkins, 1934; Loeb et al., 1933; Harrop et al., 1933). Lactation puts a heavy drain on body fluids and salts. Apparently the cortical extract dosage used in series A-I was insufficient to maintain the life of the mother and to compensate for this extraneous fluid drain of lactation. If to this dosage of extract salt solutions were added, the deficiencies in lactation were greatly alleviated. This fact indicates that the remedy of these lactation failures is not necessarily dependent upon an adrenal lactogenic hormone, but may be effected by supplying extra fluid and salt upon which small doses of the cortical hormone may act.

TABLE 4

Series A-IV: Given 2 cc. doses of cortical extract per day

(Litters of 5 treated mothers: All lived)

LITTER NUMBER	AVERAGE WEIGHT OF SUCKLING YOUNG				CHANGE IN WEIGHT OF MOTHERS DURING TREATMENT	NUMBER OF DAYS TREATED	LIFE-SPAN OF MOTHERS WHEN TREATMENT STOPPED
	On 1st day	On 10th day	On 20th day	On 30th day			
	grams	*grams*	*grams*	*grams*	*per cent*		
595	5.0	15.0	29.5	60.0	+16.0	24	45 days
596	6.5	14.5	19.7	51.0	+3.3	24	60 days
608	4.5	15.3	25.5	54.5	−1.0	26	11 days
609	5.5	17.0	25.0	56.5	−9.7	30	20 days
647	5.0	13.5	26.5	53.7	+14.9	26	Lived indef.
Average of series A-IV	5.3	15.1	25.1	55.1			
Average of normal litters	5.0	14.5	26.0	56.3			

Series A-IV. Lactation in adrenalectomized rats on large doses of cortical extract. Assuming the above interpretations to be correct, some large dose of cortical extract should support a normal lactation without salt treatment. Such was found to be the case.

A series of five pregnant rats were adrenalectomized before delivery, and given 2 cc. per day doses of cortical extract (divided doses, twice daily). The results are indicated in table 4. All litters lived. The growth rate of the young to weaning time was always within normal range.

It seems necessary to double approximately the hormone dosage essential for maintaining life to get normal lactation in adrenalectomized rats. The importance of these dosage differences is well illustrated in the isolated case shown in the protocol:

Adrenalectomized 1 day before delivery. Put on 0.3 cc. cortical extract per day (later more potent extracts—see series B–I). Young reached an average maximum weight of 12 grams on 14th day. Inadequate lactation was obvious. On 15th day one of litter died, others near death. Cortical extract dose was raised to 2 cc. per day. The next day a weight gain of 1.5 gram was noticed. From then until weaning time the slope of the growth curve was exactly parallel to normal.

Series A-V. Lactation in sub-totally adrenalectomized rats. A small piece of one adrenal left in the rat will prevent adrenal insufficiency (Pencharz et al., 1931; Gaunt, 1933, and others). It seemed probable, however, that if the amount of cortical tissue was diminished, lactation might be impaired as by inadequate hormone dosage. This was not the case as shown by the following experiment.

TABLE 5

Series A-V: Sub-total adrenalectomy

(Litters of 4 operated mothers: All lived)

LITTER NUMBER	AVERAGE WEIGHT OF SUCKLING YOUNG				CHANGE IN WEIGHT OF MOTHERS DURING LACTATION
	On 1st day	On 10th day	On 20th day	On 30th day	
	grams	*grams*	*grams*	*grams*	*per cent*
446	4.3	13.0	26.0	57.5	+29.0
447	5.3	13.5	23.0	55.0	+5.9
448	4.5	13.0	23.2	48.5	+10.5
449	6.0	18.5	33.5	60.0	+24.0
Average of series A-V	5.0	14.5	26.4	55.4	
Average of normal litters	5.0	14.5	26.0	56.3	

In four pregnant females the right adrenal was removed intact. The left one was lifted up, the blood supply blocked by holding the pedicle with curved hemostatic forceps, and at least four-fifths of the gland extirpated. The growth rate of the litters was normal, and is shown in table 5. This series of cases indicates that the surgical manipulation involved in our other experiments is not detrimental to the care of the young and the beginning of lactation. In none of these cases was adrenalectomy performed more than two days before delivery. At autopsy an enormous hypertrophy of the remaining gland fragment was noticed. This probably had occurred with sufficient rapidity to prevent lactation deficiencies.

Series A-VI. Lactation in follutein-treated females, ovariectomized five days after adrenalectomy. One obvious test of the hypothesis that there is a second hormone in the adrenal essential for lactation is to attempt to produce an experimental lactation in adrenalectomized animals. The

method of Selye, Collip and Thomson (1933) was used to induce lactation, since other methods are not reliable in the rat. These workers showed that lactation follows within two or three days after the removal of ovaries luteinized by pregnancy urine extracts. To test the effect of this procedure, the mammary glands of treated animals were removed and placed in dilute formalin (as per Selye et al.). If milk is present it clouds the formalin solution. The degree of cloudiness was used as a rough measure of the quantitative response. Milk in some cases can be squeezed from the mammae but a negative reaction in the rat is inconclusive by this test.

Twelve mature females were injected for 27 days with Follutein (Squibb). For 20 days the dose was 12.5 rat units per day; for the last 7 days, 50 R.U. per day. The adrenals were removed on the 23rd day of treatment. On the 28th day the animals were ovariectomized and tested for milk secretion during the next three days, i.e., 6 to 9 days after adrenalectomy. Of the 11 cases surviving ovariectomy, 6 lactated and 5 did not.

To help prevent the onset of adrenal insufficiency six of the animals were given salt solutions to drink after adrenalectomy. In addition, in all cases saline injections were given after ovariectomy to increase resistance against the anesthetic and surgical shock to which adrenalectomized animals are susceptible.

These results show that lactation is possible in the absence of the adrenals. The quantity of milk secreted, however, was distinctly less in all cases than in non-adrenalectomized animals. In view of the fact that in all cases following ovariectomy, adrenal insufficiency occurred, with its attendant upset in fluid balance, normal lactation could hardly be expected. It is of significance that lactation did occur in half of the cases. Those animals that had imbibed salt solutions before ovariectomy apparently lactated better than those without that extra store of fluid and salt.

Series B-I. Lactation in adrenalectomized rats treated with 0.3 cc. recent cortical extract. As mentioned before, the last ("series-B") experiments were done with cortical extracts, supplied by Doctor Swingle in 1935 and of obviously greater potency than those used previously. As seen in table 6, a lactation decidedly deficient but somewhat better than that resulting from 1 cc. per day of the earlier extracts, was obtained with 0.3 cc. per day of the recent materials. A normal lactation probably would have occurred with 1 cc. or less per day. Four of six litters lived but growth in no case was entirely normal. The mothers were maintained in good condition. The lactation-promoting qualities of the extracts would seem then to be gauged by their cortical hormone potency.

Series B-II. Lactation in adrenalectomized rats treated with 0.3 cc. cortical extract plus prolactin. One possible explanation of the lactation failures found in our previous experiments was that adrenalectomy in-

hibits the normal activity of the pituitary. Considerable evidence (Martin, 1932; Grollman and Firor, 1935) indicates a pituitary dysfunction after adrenalectomy.

This possibility was tested by using a dose of cortical extract which would not support a normal lactation (0.3 cc. per day) plus injections of prolactin (Squibb)—10.3 bird units per day—in rats adrenalectomized just before delivery. Results with six litters are given in table 6. Four rats raised their litters and two did not. The growth rate was almost exactly the same as that obtained with 0.3 cc. of cortical extract without prolactin. In another case prolactin injections were begun on the 15th day of lactation in an animal that was lactating sub-normally on 0.3 cc. cortical extract. No improvement was observed.

It was concluded that no deficiency in prolactin caused the lactation failures observed in other experiments.

TABLE 6

Effects of cortical extracts and pituitary extracts

SERIES	TREATMENT	SURVIVAL OF LITTERS	AVERAGE WEIGHT OF SURVIVING, SUCKLING YOUNG			
			On 1st day	On 10th day	On 20th day	On 30th day
			grams	*grams*	*grams*	*grams*
B-I	0.3 cc. Cort. Ext.	4 lived; 2 died	5.6	11.1	17.2	33.2
B-II	Cort. Ext. & Prolactin	4 lived; 2 died	5.1	10.6	18.1	36.9
B-III	Cort. Ext. & Ant. Pit.	1 lived; 5 died	5.0	7.8	16.6	36.0
Normal stock litters			5.0	14.5	26.0	56.3

Series B-III. Lactation in adrenalectomized rats receiving 0.3 cc. cortical extract and anterior pituitary extract (Squibb). Grollman and Firor reported that growth and other deficiencies in rats with chronic adrenal insufficiency were remedied by anterior pituitary extracts. It seemed possible, therefore, that the Squibb pituitary extract (assayed and marketed for its content of growth hormone) might supplement the sub-minimal doses (from the standpoint of lactation) of cortical extract.

Under conditions the same as described in series B-II, 1 cc. per day of anterior pituitary extract (Squibb) was added to 0.3 cc. daily doses of cortical extract. Results are shown in table 6. Of six rats treated only one raised its litter. The pituitary extract obviously inhibited lactation, as compared to the cortical extract treatment alone. This was probably because of its gonadotropic content.

DISCUSSION AND CONCLUSIONS. Normal lactation would not be expected in untreated, adrenalectomized animals because of the generally

fatal complications resulting from adrenal insufficiency. The work of Carr, using an extract made by the Swingle-Pfiffner method, indicated that normal lactation does not occur in adrenalectomized rats even when life is maintained by administration of cortical extract. Brownell et al. got similar results with the Hartman extracts (13 per cent of young lived). Our first set of experiments (series A-I) seemed to confirm this observation. Later experiments indicated, however, that the deficiency was one of dosage only. If enough cortical extract is given lactation will be approximately normal. In the Swingle-Pfiffner cortical preparations nothing essential for lactation is lacking. The results of Brownell et al. were interpreted as indicating that a separate, lactogenic hormone was present in the adrenal which is ordinarily discarded in their cortical preparations. This lactation hormone, when added to that sustaining life, supported lactation only to the extent of keeping 51 per cent of the young alive in one series and 65 per cent in another. Obviously something still was missing. Our results would indicate that the relief from lactation failures after adrenalectomy is to be provided by the same things which remedy adrenal insufficiency most successfully, namely, cortical hormone and salt treatment. The variable of apparent prime importance is that of unitary dosage. A treatment by salt or by *extract sufficient only to keep an adrenalectomized mother in fairly normal condition will not be sufficient to support a normal lactation.* The fluid and electrolyte drains involved in the secretion of milk require, we presume, an extra amount of hormone. It is of interest to recall that in the guinea pig, at least, there is a hypertrophy of the adrenal during pregnancy and lactation (Verdozzi, 1918; Castaldi, 1922).

The fact that some rats, which can be made to consume large quantities of salt solution, lactate fairly normally on that treatment alone, and that an experimental lactation can be produced in some rats in the complete absence of the adrenals, does not indicate the presence of a lactogenic hormone of adrenal origin.

The correlation of these data with those offered by Brownell et al., if the dosage variable is not adequate for such correlation, must await further investigation. The discrepancies could be due in part to the different types of extract used. The nature of the variations, however, does not suggest that explanation. Another variable between the work of Brownell et al. and ours was that of the type of rat used. They reported that only 72 per cent of their normal young were raised. Some non-experimental factor, perhaps a dietary deficiency, may have been inhibiting lactation.

SUMMARY

1. Adrenalectomized rats treated with an adequate life-maintaining dose (1 cc. per day) of the earlier Swingle-Pfiffner cortical extracts raised their litters in only 4 out of 10 cases. The growth of the surviving young was sub-normal (series A-I).

2. The results with adrenalectomized rats given salt treatment were similar to those given extract treatment (series A-II).

3. A combined treatment of cortical extract plus salt in adrenalectomized rats resulted in a lactation that approached but did not quite reach normal (series A-III).

4. When the doses of cortical extract were raised to 2 cc. per day (an amount more than twice that necessary to keep the adrenalectomized mothers alive) all litters lived and lactation was normal as judged by the growth of the young (series A-IV).

5. During lactation the cortical hormone dose must be approximately doubled, presumably to accommodate for the fluid and electrolyte drains of milk secretion.

6. Sub-total adrenalectomy did not influence lactation (series A-V).

7. An experimental lactation was produced in half of a series of totally adrenalectomized rats (series A-VI).

8. When extracts of increased cortical hormone potency were used, their effectiveness in supporting lactation was likewise increased (series B-I).

9. The sub-normal lactation accompanying life-maintaining doses of cortical extract is not remedied by prolactin (series B-II).

10. The addition of anterior pituitary extract (Squibb) to a sub-minimal dose of cortical extract definitely inhibited lactation (series B-III).

11. These experiments offer no evidence in support of the hypothesis that there is a second hormone in the adrenal cortex, separate from the life-maintaining hormone, which is essential for lactation.

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DEVELOPMENT OF THE FETAL RAT FOLLOWING ELECTRO-CAUTERY OF THE BRAIN

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In a preliminary paper observations were reported on the effects of brain cautery on growth and behavior in an extended series of rat fetuses (Corey, 1934). The results indicated that weight and crown-rump length of the operated animals were approximately 10 per cent less than in their litter-controls. There were no noteworthy differences in muscular, respiratory and other responses. In these experiments (on 70 fetuses) the operations were carried out at various gestational ages, and it appeared possible that more significant results might be obtained if the conditions were more rigidly standardized. The present paper is a complete report of earlier as well as more recent studies.

METHODS. Albino rats of the Wistar Institute colony, varying between 108 and 150 days old, were used throughout the recent study. The time of insemination was accurately recorded in every mating, and the age of all fetuses calculated as from that time. Operations were performed on the 16th (6 litters) or 17th (35 litters) day of gestation, and all fetuses were removed from the uterus on the 21st day (at term). The average number in the litters was 8.7 fetuses. Operations were performed under ether anesthesia, and each fetus was transuterally cauterized just posterior to the frontal-parietal junction in the mid-line. Three fetuses were thus treated in each instance, their litter-mates serving as controls. It had previously been shown in a series of control experiments that extensive cautery of one fore-limb produced no significant effect on subsequent general growth and behavior. Care was exercised to standardize the procedure as far as possible, but the difficulty of restricting the extent of brain injury in such very small organisms to certain limits will be readily apparent. Fetuses recovered from the uterus were tested for muscular activity and other reactions, weighed, and placed in 5 per cent formalin preparatory to microscopic study of the injured area.

RESULTS. In 10 litters the operated fetuses died before term, and there was one instance of maternal death following operation. In the remaining 30 litters, 50 out of 90 (55 per cent) brain-cauterized fetuses were recovered alive at term.

Gross appearance. The living experimental fetuses recovered from the uterus presented certain distinct gross abnormalities, namely, a shortened rostral region (the maxilla being more markedly affected than the mandible), enlarged and protruding tongue, and heavy neck and jaws. There was no evidence of tissue regeneration or repair in the cauterized area in any instance, although the general and cerebral vascularity in all cases appeared relatively unimpaired. The abnormal appearances described were almost invariably confined to the head region and may probably be ascribed to mechanical effects, produced by cautery alone.

Behavior. Muscular movements exhibited by cauterized fetuses were little different from those seen in litter-controls, except that in nearly all cases they appeared somewhat weaker and more sluggish. Of the 50

TABLE 1

Summary of results obtained on 90 cauterized fetuses comprising 51 litters

SERIES I, 21 LITTERS	CONTROL (28 FETUSES) WEIGHT	EXPERIMENTAL (40 FETUSES) WEIGHT	DIFFERENCE
	grams	*grams*	*per cent*
Whole fetus	4.30	3.80	10
	mm.	*mm.*	
C-R length	39	36	8
SERIES II, 30 LITTERS	**CONTROL (150 FETUSES) WEIGHT**	**EXPERIMENTAL (50 FETUSES) WEIGHT**	**DIFFERENCE**
	grams	*grams*	*per cent*
Whole fetus	3.90	3.39	13
Fetal head	0.95	0.79	15
Fetal body	2.95	2.60	11
	mm.	*mm.*	
C-R length	34	31	9

fetuses observed, all exhibited active muscular movements, 44 made definite respiratory movements and 27 fetuses were capable of vocalization (squeaking).

Growth. Findings on the growth-rate of operated fetuses are summarized herewith. In 5 cases (series II) the experimental fetuses exceeded the controls in weight, and in 3 cases equalled or exceeded their littermates in length. In a total of 51 litters, therefore, the experimental fetuses were found to be smaller in weight and length than their litter-controls by 12 and 9 per cent respectively. These differences were apparently insignificant.

Neurological findings. Microscopic examination revealed wide variation in the extent of brain disorganization following cautery. Such

damage varied from slight cortical injury to extensive disorganization involving the entire brain to a level below the pons.

From the table herewith it appears that differential destruction of the cortical areas of the brain is not correlated with varying degrees of muscular activity. A slight reduction in respiratory rate and a pronounced decrease in the percentage of fetuses exhibiting vocalization occurred, however, in the "decerebrate" group. Analysis of each case revealed that complete disorganization above the optic chiasma resulted in no pronounced differences in the behavior of cauterized fetuses.

Weight discrepancies between experimental fetuses and their litter-controls could not be correlated with the extent of brain injury. Thus in 9 litters in which all operated fetuses were of the "decorticate" class, 3 of the experimental animals weighed more than the average of their controls. The average body weight of all of the operated fetuses in these 9

TABLE 2

Reactions in "decorticate" and "decerebrate" rat fetuses

	NUMBER OF CASES	MUSCULAR MOVEMENT	RESPIRATION	VOCALIZATION
"Decorticate" (partial to complete cortical destruction or disorganization)	15	15 (100%)	15 (100%)	12 (80%)
"Decerebrate" (complete cortical destruction with slight thalamic involvement, to complete disorganization to below level of pons)	35	35 (100%)	29 (83%)	15 (43%)

litters was 13 per cent less than for their litter-mates. Again, in 6 litters in which all operated fetuses were classified as "decerebrate," the average body weight of the cauterized fetuses of 2 litters was greater than that of their controls, and the body weight of all operated fetuses averaged 13 per cent below the controls.

Discussion. That the mammalian fetus may survive a drastic operative procedure involving destruction of 10 to 75 per cent of the total brain tissue is of itself noteworthy. That the destruction of such highly differentiated structures is followed by no pronounced untoward effect on subsequent embryonic growth and development appears particularly significant from the standpoint of fetal physiology and behavior. The results argue strongly for absence of function or hypofunction of the higher nervous centers during fetal life. The consideration that many of the fetal functions are regulated through the maternal circulation and the surrounding media (e.g., placenta) makes the results appear, however, rather less surprising.

The anencephalic (and microcephalic) human fetus commonly reaches term, it is said, with its growth-rate but little altered. Nanagas (1925), in what is perhaps the most extensive statistical study of this condition, found that 96.2 per cent of such fetuses were somewhat below the normal in weight. In series II of the present paper, 42 out of 50 animals (84 per cent) were below the average control weight, but the discrepancies were on the whole slight. Marcus and Nickman (1930) cite a typical instance of anencephaly, and state that such children are "able to swallow, make chewing movements, cry and respond to irritation . . .".

Hooker and Nicholas (1930) found that rat fetuses in which spinal section had been performed developed normally, and that their behavior at term was similar to that of litter-controls, with the exception of some sluggishness of movement. Absence of regeneration at the site of brain injury in the present experiments is in agreement with the findings in amputation experiments (Nicholas, 1926) on the embryonic fore-limb (rat).

Functional studies by others on adult rats offer evidence which is in agreement with the present experiments. Thus Lashley (1921) observed that destruction of the motor cortex produced no discernible disturbances in behavior, but that paralysis resulted if the corpus striatum were included in the destroyed area. Herrick (1926) states that cortical differentiation in such primitive placentals as the rat is "still in its incipiency."

In a posthumous review by Huber (1934) it is stated that "the excitable cortical centers of rodents appear to be ill defined and variable." This lack of definition in the cortex of the adult rat would lead one to expect an even greater lack of functional differentiation in the fetus. Vagal cardiac control, it may be observed, has been shown to be "absent or poorly developed" in the fetal dog (Clark, 1932). On the other hand, cardiac sympathetic function appears to be well established in the rat before birth, and there are many evidences also of vagal control being present (Corey, 1935). Inability to rear cauterized animals which were born alive (12), perhaps due to trauma of the fetal wound by the mother and the failure of artificial feeding, renders it impossible to determine the necessity of the brain centers in early post-natal life.

SUMMARY

The fetal rat survives in apparently normal condition throughout the last quarter of the gestation period following destruction of 10 to 75 per cent of the brain tissue involving levels well below the pons. Certain anatomical abnormalities observed on the removal of fetuses from the uterus were attributed to mechanical factors produced by cauterization. Muscular responses were sluggish but otherwise indistinguishable in character from those observed in litter-controls.

The experimental fetuses were smaller in average weight and crown-

rump length than their litter-mates by 12 and 9 per cent respectively. These differences are not statistically significant.

Neurological examination revealed that different degrees of fetal brain destruction above the level of the optic chiasma were not correlated with muscular activity exhibited at term or the weight discrepancies noted above.

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THE ACTION OF THE NITROGENOUS BASES OF THE GASTRIC JUICE ON BLOOD PRESSURE, PANCREATIC SECRETION AND FLOW OF BILE

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Canine gastric juice was found to contain considerable amounts of nitrogenous bases precipitable by phosphotungstic acid. The substances of this fraction, which as carbonates are soluble in absolute alcohol, exhibited a secretagogue action on the gastric glands when administered subcutaneously or intravenously. A considerable part of this secretory property was found to be recoverable in the histidine-arginine fraction, the purine and lysine fractions being practically inactive, even when injected in very large doses (Komarov, 1933). It was also demonstrated that vitamin B_1 is a constituent of canine gastric juice (Komarov, 1934). Its presence could be shown in the fraction isolated from the nitrogenous bases of the gastric juice by precipitation with $AgNO_3$ and $Ba(OH)_2$ at pH 7.0.

These investigations showed that some of the nitrogenous bases of the gastric secretion are physiologically active bodies. A further study of the influence of these bases on the circulatory system and on the other digestive glands is reported in the present paper.

METHODS. The histidine, arginine and lysine fractions used in the experiments described below are isolated from 9 liters of gastric juice, which had been obtained by means of sham-feeding from three dogs, each having esophagotomy and a gastric fistula. The daily secretion of about 800 cc. was immediately filtered, neutralized with Na_2CO_3 and the same day concentrated under reduced pressure to one-tenth the original volume. This product was kept under toluene at 3°C. until the necessary amount was accumulated. The reaction was adjusted to pH 2.8, and the voluminous precipitate was separated by centrifuging, washed with dilute HCl (pH 2.8) and finally with a small amount of distilled water. The supernatant liquid and washings were neutralized with NaOH and treated with lead acetate (350 cc. of 33 per cent solution). After standing overnight the precipitate was filtered off and washed with distilled water. The filtrate and the washings were treated with a slight excess of baryta. The precipitate formed was removed within one hour by suction, the filtrate being immediately neutralized with sulphuric acid. The new filtrate was con-

centrated to 200 cc. and from it the fraction of the nitrogenous bases was prepared by precipitation with phosphotungstic acid (for details, see Komarov, 1933). The nitrogenous bases were further fractionated by the silver-baryta method. A precipitate obtained by precipitation with silver nitrate at pH 2.8 (purine fraction) was discarded. The second silver precipitate, obtained by adding baryta up to pH 7.0, was decomposed with HCl and is hereinafter designated as the "histidine fraction." The third silver precipitate was obtained by adding baryta under ice-cooling until no more precipitate was formed. The precipitate was separated within fifteen minutes by centrifuging, then immediately suspended in water and decomposed with HCl. This fraction is hereinafter referred to as the "arginine fraction." The "lysine fraction" was prepared from the filtrate after the third silver precipitate (for details, see Komarov, 1933). Only those bases of the lysine fraction, which as carbonates and as chlorides were easily soluble in absolute alcohol, were studied in this investigation.

The experiments were carried out on young dogs of 5 to 7 kgm. weight, which for twenty-four hours before the experiment had received water but no food. They were anesthetized with morphine (0.01 gm. per kgm. weight), more complete narcosis being secured when necessary by injection of chloralose. The pancreatic duct and bile ducts were cannulated. The gall bladder was extirpated in a number of experiments. The pylorus was obstructed by means of a ligature passed between the muscular and submucous layers. Blood pressure was recorded from the carotid artery. The test solutions were injected into the femoral vein.

EXPERIMENTAL RESULTS. *Histidine fraction.* The histidine fraction contained 0.2 mgm. per cent of nitrogen as calculated for the original gastric juice. This fraction, when injected intravenously in doses corresponding to from 360 to 600 cc. of gastric juice, produced no effect either on blood pressure or on pancreatic secretion or flow of bile.

Arginine fraction. The arginine fraction contained 3.90 mgm. per cent of nitrogen, as calculated for the original gastric juice.

a. *Effect on blood pressure.* When injected in amounts corresponding to from 300 to 500 cc. of gastric juice, this fraction caused in all the experiments a sharp fall of blood pressure (fig. 1) in every respect similar to that produced by injection of about 0.1 mgm. of histamine. This depressor effect was not abolished by preliminary administration of 5 mgm. of atropine.

b. *Effect on pancreatic secretion.* A definite activation of the pancreatic secretion was observed in all the experiments. The slight continuous secretion due to the action of morphine was markedly accelerated by the injection of solutions of the arginine fraction in amounts corresponding to from 270 to 500 cc. of gastric juice. The latent period was about one

minute, and the effect was of rather short duration, lasting only a few minutes. Preliminary administration of atropine (1 mgm. per kgm., weight) did not abolish the secretory effect.

The experiment of June 20, 1934, is quoted as a typical example. The pancreatic secretion, noted every five minutes in divisions of graduated tubing, was as follows:

Spontaneous: 4.
After intravenous injection of 5 cc. of the arginine fraction (= 425 cc. of gastric juice): 15, 4, 3, 4, etc.
After administration of 5 mgm. of atropine sulphate:
Spontaneous: 4.
After intravenous injection of 6 cc. of the arginine fraction (= 510 cc. of gastric juice): 20, 5, etc.

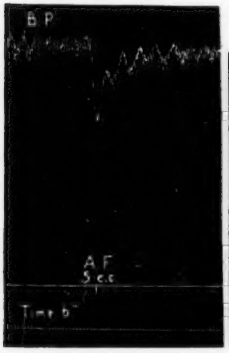

Fig. 1

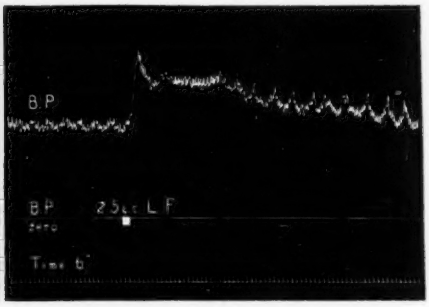

Fig. 2

Fig. 1. Experiment 4 (June 14, 1934). Dog, 7.0 kgm. Injection of 5 cc. of the arginine fraction (corresponding to 540 cc. of gastric juice) produced a sharp fall of blood pressure, the effect lasting about 3 minutes.

Fig. 2. Experiment 6 (July 23, 1934). Dog, 6.0 kgm. Vagi cut. Injection of 2.5 cc. of the lysine fraction (corresponding to 450 cc. of gastric juice) exerted a marked and prolonged pressor effect.

c. *Effect on the flow of bile.* Administration of the arginine fraction increased the flow of bile in a few experiments in which the gall bladder had been left intact and the solution was injected at the beginning of the experiment. No such effect was observed when the fraction was administered after the experiment had been in progress for two or three hours. Furthermore, in animals in which the gall bladder had been extirpated, and in which it was thus possible to study the true biliary secretion as opposed to the discharge of bile, the arginine fraction was without any marked effect on the secretion.

Lysine fraction. This fraction contained 3.52 mgm. per cent nitrogen as calculated for the original gastric juice. The Sakaguchi (1925) reaction for the derivatives of guanidine was positive.

a. *Effect on blood pressure.* The effect of intravenous administration of this fraction on the blood pressure was found to depend on the dose used and on the initial level of the blood pressure. In certain cases in which the dose given was relatively small (equivalent to 360 cc. of gastric juice) and the blood pressure initially fairly high (85 mm. Hg), no noticeable effect could be observed. The action of larger doses, corresponding to from 400 to 900 cc. of juice, was on the other hand very striking: the blood pressure rose sharply to a peak, which was in all the experiments at about the same level, *viz.*, 140 to 145 mm. Hg, and then returned slowly to its original level. The duration of the rise varied between 15 and 30 minutes in different experiments, depending on the quantity of the extract injected. The absolute increase in blood pressure was greater when the initial level was comparatively low. The pressor effect of the lysine fraction was not abolished by preliminary section of the vagi (fig. 2) or by administration of atropine (5 mgm.).

b. *Pancreatic secretion.* A positive secretory effect, following intravenous injection of the lysine fraction, in doses corresponding to from 360 to 900 cc. of gastric juice, was noted chiefly in those experiments in which the blood pressure rise was insignificant or even absent. As is known, the rise in blood pressure due to vasoconstriction is very unfavorable to the secretory activity of the pancreas. As an example the experiment of June 26, 1934, is quoted, in which two small doses of the lysine fraction were introduced. No effect on the blood pressure was observed. The pancreatic secretion was noted every five minutes in divisions of graduated tubing, and was as follows:

Spontaneous: 8, 7, 7.
After intravenous injection of 4 cc. of the lysine fraction (= 360 cc. of gastric juice): 10, 12, 7, 6, 7, 5, 5, 5.
After 2nd injection of 4 cc. of the lysine fraction: 9, 7½, 7½, 7½, 4½.

The secretagogue action of the lysine fraction also remained unaffected after administration of atropine.

c. *Secretion of bile.* In all the experiments where the gall bladder was extirpated, the lysine fraction, in doses corresponding to from 400 to 900 cc. of gastric juice, accelerated the biliary secretion, in some cases very strikingly. The effect was not abolished by section of the vagi or by atropinization. The latent period was 1 to 2 minutes, and the duration of the action 15 to 40 minutes.

The experiment of July 5, 1934, may be taken as typical. The gall bladder was extirpated. The secretion was read every five minutes in divisions of graduated tubing.

Spontaneous: 9, 9, 9.
After intravenous injection of 4.5 cc. of the lysine fraction (= 405 cc. of gastric juice): Rise of blood pressure; secretion, 12, 30, 30, 30, 20, 20, 18, 18, 12, 8, 10, 8.

It should also be mentioned that administration of the lysine fraction was accompanied as a rule by general movements of the body and sometimes even by convulsions.

Discussion. The histidine fraction, which has been shown to exert a marked curative effect on polyneuritic pigeons (Komarov, 1934), did not affect the blood pressure or the pancreatic or biliary secretion in the dog. This fact indicates that the anti-neuritic properties of the fraction cannot be attributed to the presence of non-specific substances like histamine. As a concentrate of vitamin B_1, the histidine fraction of gastric juice is in every respect similar to the highly purified preparations studied by Anrep and Drummond (1919–20) and by Guha (1931), so far at least as concerns their action on blood pressure and on pancreatic secretion.

The arginine fraction on intravenous injection caused a sharp fall in blood pressure and a definite acceleration of the pancreatic secretion, with no effect on the flow of bile. None of these actions was to any degree influenced by preliminary atropinization. Previously it was demonstrated (Komarov, 1933) that the histidine-arginine fraction also activates gastric secretion. Of all the substances which, if present in gastric juice, would be recoverable in the arginine fraction, only histamine (or some unknown body chemically very similar to it) could produce all the effects observed. In fact, the effect of the arginine fraction on the blood pressure and on the secretion of pancreatic juice could be very closely imitated by histamine in suitable dosage (0.1 mgm. of histamine being approximately equivalent to the arginine fraction corresponding to 400 cc. of gastric juice). A more detailed pharmacological study of this extract is necessary, however, before the active principle can be regarded as identical with histamine; for final identification, of course, only the actual isolation of the substance responsible would suffice.

After the experimental part of this work had been completed and incorporated in a Ph.D. thesis (Komarov—McGill University, May, 1935), a paper was published by Brown and Smith (1935), in which these authors reported the presence of a histamine-like substance in the human gastric juice. However, their attempts to isolate histamine chemically from gastric juice were unsuccessful.

Administration of the lysine fraction in sufficient amount produced a marked rise of blood pressure, the whole effect being of long duration. In most cases it increased the secretion of pancreatic juice and of bile. Definite conclusions as to the nature of the substance or substances responsible are not justified. Certain pharmacodynamic effects of this fraction, however, when considered in connection with the positive Saka-

guchi reaction, are suggestive of the presence of certain derivatives of guanidine. Some of these substances, especially methyl- and dimethylguanidine, are known to possess a manifold pharmacodynamic activity, evoking typical convulsions, prolonged elevation of the blood pressure of peripheral origin (Bovschyk and Sinelnikov, 1916), and stimulation of most of the secretions (Komarov, 1924; Krimberg and Komarov, 1926). Convulsions were frequently observed to follow the injection of larger doses of the lysine fraction in the experiments described. The hypothesis that guanidine or its derivatives are normal constituents of the gastric secretion would only be soundly substantiated by actual isolation of these substances by methods excluding the possible formation of methylguanidine through oxidation of creatinine.

SUMMARY

1. The histidine fraction isolated from canine gastric juice produced no effect on blood pressure, pancreatic secretion or flow of bile.

2. The arginine fraction exhibited a histamine-like action on the blood pressure and the secretion of pancreatic juice. It had no definite effect on the secretion of bile.

3. The lysine fraction possessed a marked pressor activity, and stimulated the secretion of pancreatic juice and of bile. None of these effects were influenced by preliminary section of the vagi or by atropinization.

The writer gratefully acknowledges the very helpful criticisms and advice which he has received from Dr. B. P. Babkin during the course of this work.

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BRAIN WEIGHT AND MOISTURE CONTENT IN NORMAL CHICKS AND THOSE WITH NUTRITIONAL ENCEPHALOMALACIA

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Upon a synthetic diet[1] which contained all of the factors known to be necessary for normal rat growth, young chicks developed in the brain a severe disorder, preventable by the addition of the non-saponifiable fraction of soy bean oil (1, 2, 3, 4). In this disease, to which the term nutritional encephalomalacia has been given, the essential pathological change is an ischemic necrosis of the cerebellum. Cerebrum, mid-brain, and medulla are less severely affected, optic lobes, spinal cord, and peripheral nerves not at all. Constant visceral lesions have never been noted (5).

The insusceptibility of older chicks to nutritional encephalomalacia made it probable that the disease occurred only during the period of active brain growth. Furthermore, it was thought that the difference in susceptibility to injury between the cerebrum and cerebellum might be associated with a difference in their respective rates of growth. For these reasons a series of weight determinations of brain at different ages was made.

Donaldson (6) showed many years ago that in the rat the coefficient of correlation between the weight of the brain and body weight was higher than that between brain weight and age. Moreover, he showed that this relationship is not significantly modified either by sex or by the rate of growth of the animal. Later, Stewart (7) and Donaldson and Hatai (8)

[1] Diet 108 has the following composition:

	per cent
Skimmed milk powder (Merrell-Soule)	15.0
Casein (technical)	20.5
Cornstarch	20.0
Lard	21.0
Cod liver oil (Mead Johnson Co.)	2.0
Yeast (Northwestern, powdered, non-irradiated)	5.0
Salt mixture (McCollum 185)	6.5
Paper pulp (Eastman)	10.0

found that changes in the rate of growth are the same for the different parts of the brain. From data presented in this paper it will be seen that similar conclusions may be drawn with respect to the relationship between weights of the whole brain, cerebrum, cerebellum, and body weight in the chick.

One of the obvious pathological changes observed in the brain of chicks with acute lesions of nutritional encephalomalacia is edema. It will be shown that there is an actual increase in the moisture content of the affected parts of the brain.

For purposes of comparison with edematous brain, the moisture content of the normal cerebrum, cerebellum, mid-brain and spinal cord of the chick was determined. The values were found to be in general agreement with those obtained by Haldi, Larkin, and Wright (9) in the rabbit. Within the age period studied, age was found to be without influence upon the percentage moisture, an observation which was unexpected because of the demonstration by Donaldson (10) and Donaldson and Hatai (8) that in the growing rat the percentage moisture decreases with age.

EXPERIMENTAL PROCEDURE. The brain weight and moisture data were collected in the course of dietary experiments upon White Leghorn chicks. Normal brain weight observations were made upon 112 chicks killed at various ages from hatching to 12 weeks. Of these, 82 were on stock diet 634 of Hogan, Hunter and Kempster (11)[2] and 30 on diet 108. Normal moisture determinations were carried out upon the brain of 118 chicks, 38 of which were on diet 634 and 80 on diet 108 and modifications. Thirty-six chicks on diet 108 and modifications with nutritional encephalomalacia were studied.

Chicks were placed from the time of hatching either upon the disease-producing diet 108 and modifications or upon the control diet 634. On the development of symptoms characteristic of cerebellar disorder, usually in the third and fourth weeks, or at the termination of the experimental period after five to eight weeks, the chicks were chloroformed. The brain was removed as soon as possible, separated into parts, weighed, and divided into two portions, one being reserved for pathological study and the other

[2] Diet 634 of Hogan, Hunter, and Kempster:

	per cent
Whole wheat	55.6
Whole milk powder	8.2
Casein	12.3
Alfalfa meal	2.5
Butter fat	4.2
NaCl	0.9
$CaCO_3$	1.3
Cod liver oil	3.0
Yeast	12.0

used for moisture determinations. Since the separation of the medulla from the spinal cord was not accurately made at the same level, the weight of the whole brain is subject to greater error than that of the cerebrum and cerebellum.

The amount of moisture was determined by heating at 100°C. rather than by the more approved but tedious method of drying the tissue at room temperature in a vacuum over sulfuric acid. In a comparison of the two methods, Donaldson (10) found no significant difference between

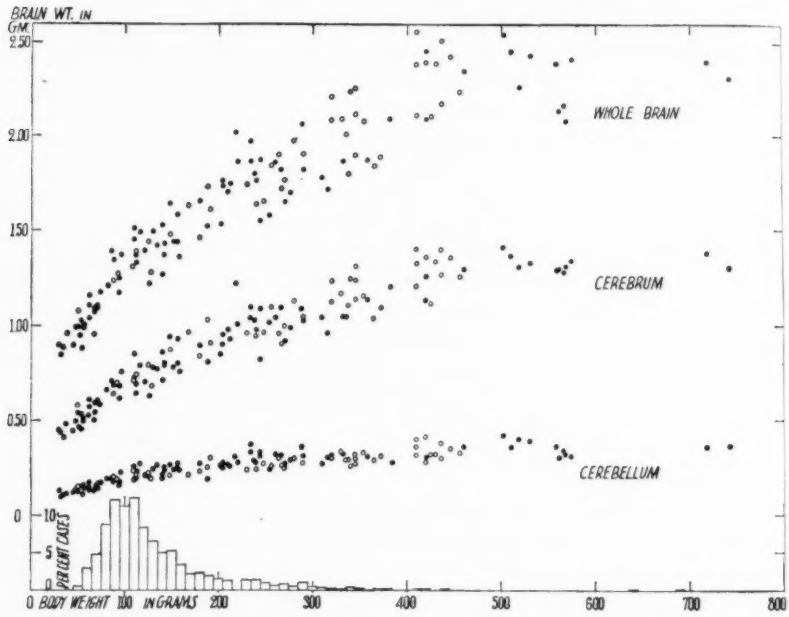

Fig. 1. Showing the relationships between *a*, the weights of the different parts of the brain and gross body weight in normal White Leghorn chicks, and *b*, the rate of brain growth and the occurrence of nutritional encephalomalacia. Dots represent data from chicks on diet 634 and circles, those on diet 108.

them. Heating at 100°C. for 3 hours was found to be sufficient to bring the tissue to constant weight, but for routine determinations a 20 hour period was adopted. Small glass stoppered weighing bottles were used. Samples of cerebrum taken for analysis weighed approximately 0.3 gram; cerebellum, 0.1 gram; mid-brain including medulla and optic lobes, 0.08 gram; and spinal cord, 0.1 gram. Duplicate determinations gave an average difference of 0.6 per cent moisture.

Relationship between weight of the brain and gross body weight. The re-

lationship between the weights of the different parts of the brain and gross body weight is shown graphically in figure 1. The weights of cerebellum, cerebrum, and whole brain are plotted against gross body weight rather than age on account of the better correlation obtained. As sex was found to be without significant influence, the records of males and females are not treated separately. The brain weights of the chicks on diet 108 are represented by circles, those on diet 634 by solid dots. Although chicks on diet 108 grew more slowly than those on diet 634 (2), it is seen that the relationship between weight of the brain and body weight was not altered.

From the chart it is apparent that the different parts of the brain reach their maximum weight simultaneously at the time when the chick weighs approximately 550 grams. On an optimum diet this body weight corresponds to an age of 9 weeks. During the first three weeks, in which the normal chick attains a mean body weight of 150 grams, the rates of growth of the brain are greater than in the succeeding 6-week period. There is no obvious difference in the rate of growth between the cerebrum and cerebellum.

In order to compare the rate of brain growth with the occurrence of encephalomalacia the frequency distribution curve of the maximum body weights attained by 761 chicks with the disease was plotted and also included in the chart. The data from all affected chicks on diet 108 and modifications were used regardless of the type of brain lesions, sex, or length of preliminary period on diet 634. Consideration must, however, be given to the influence of the arbitrary termination of the dietary experiments at 40 days, a procedure which reduced somewhat the probability of the occurrence of the disease at body weights above 150 grams.

It is evident that encephalomalacia occurs during the period of active growth of the brain. Seventy-five per cent of the chicks were found to develop brain lesions before they reached a body weight of 150 grams. Only 2 out of 761 chicks with lesions weighed more than 550 grams at autopsy. One presented marked healed lesions, and the other one had been kept on diet on 634 for 26 days before changing to diet 108.

Brain weight in encephalomalacic chicks. The weights of cerebellum and cerebrum of 33 chicks with encephalomalacia are plotted in figure 2. Circles represent parts of the brain with lesions and large dots, the parts that remained normal. Small dots represent normals as given in figure 1. In addition to brain weights the chart shows one of the characteristic features of the disease, namely, that the cerebellum is more commonly affected than the cerebrum.

It is seen that there is considerable variation in brain weight when lesions are present, although usually the weight of the affected parts is increased. This is due to the differences in the character of the lesions at different stages of the disease.

The cerebellum in chicks which are killed soon after the appearance of the symptoms is found to be greatly swollen, softened, and edematous; often there are minute hemorrhages visible on the surface. The convolutions are flattened. In older lesions, sometimes found in chicks that showed no symptoms, the necrotic areas take on a greenish-yellow, opaque appearance. Still later, if the chick survives, and opportunity for healing has been given, the affected lobules become shrunken, depressed below

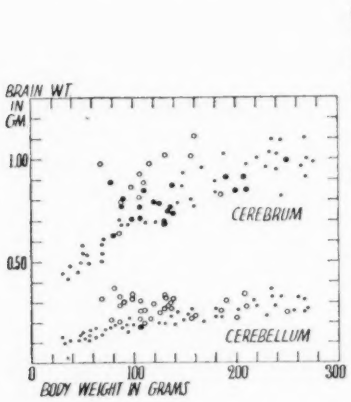

Fig. 2

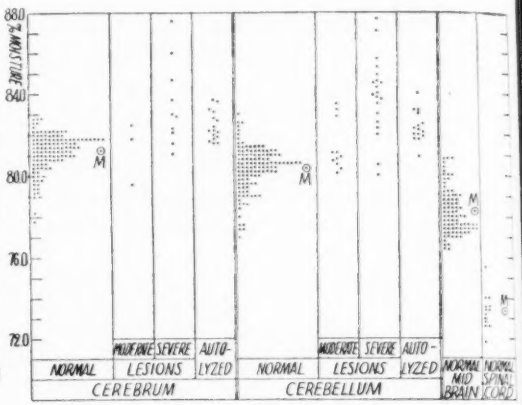

Fig. 3

Fig. 2. Showing the brain weights of encephalomalacic chicks. Circles represent parts of the brain with lesions; large dots, parts that remained normal; and small dots, normal weights.

Fig. 3. Illustrating moisture content of different parts of the brain of normal White Leghorn chicks and those with nutritional encephalomalacia.

Normal cerebrum: 118 determinations; mean, 81.27 ± 0.04; standard deviation, 0.67 ± 0.03; coefficient of variation, 0.80 ± 0.03.

Normal cerebellum: 118 determinations; mean, 80.37 ± 0.07; standard deviation, 1.10 ± 0.05; coefficient of variation, 1.30 ± 0.05.

Normal mid-brain: 83 determinations; mean, 78.27 ± 0.07; standard deviation, 1.00 ± 0.05; coefficient of variation, 1.30 ± 0.07.

Normal spinal cord: 13 determinations; mean 73.39 ± 0.19; standard deviation, 1.09 ± 0.14; coefficient of variation, 1.40 ± 0.18.

the surface of the healthy tissue, and brownish-yellow in color. The extent of the lesions varies from case to case; as much as four-fifths of the entire cerebellum may be affected; at the other extreme are lesions so small that they can not be recognized with the unaided eye.

In the cerebrum one or both hemispheres may be affected. The necrotic tissue is often extremely pale, swollen, and wet; it soon becomes sharply delineated from the normal tissue. Again, there are individual

differences in the extent of the lesions. In extreme cases the greater portion of both hemispheres may be destroyed; on the other hand, small lesions may be disclosed only under the microscope.

Increase in moisture content of the encephalomalacic brain as compared with the normal. The results of the moisture determinations of 118 chicks of both sexes with normal brain are plotted in figure 3 and described in

TABLE 1

Showing the percentage moisture in the brain of 14 to 35 day-old chicks on diet 108 and modifications, affected with nutritional encephalomalacia

CEREBELLUM		CEREBRUM		MID-BRAIN	
Per cent moisture	Extent of lesion	Per cent moisture	Extent of lesion	Per cent moisture	Extent of lesion
87.76	Severe	81.83	Normal	77.59	Normal
87.20	Severe	81.55	Normal		
84.86	Severe	80.94	Normal	78.18	Normal
84.53	Severe	80.69	Normal		
84.50	Severe	81.43	Normal		
84.39	Severe	80.42	Normal		
84.05	Severe	81.56	Normal		
83.90	Severe	84.72	Severe	78.94	Normal
83.18	Moderate	81.47	Normal	77.75	Normal
82.92	Moderate	81.00	Normal		
82.91	Severe	81.91	Normal	78.84	Normal
82.58	Severe	82.94	Severe	77.96	Normal
82.26	Severe	83.00	Severe	77.23	Normal
81.08	Moderate	83.77	Severe		
81.08	Moderate	80.89	Normal		
80.61	Moderate	80.67	Normal		
80.58	Moderate	80.58	Normal	77.80	Normal
80.50	Severe*	79.60	Moderate*	77.89	Normal
80.44	Normal	81.79	Moderate		
80.17	Moderate	82.06	Severe		
Normal mean					
80.37		81.27		78.27	

* Late stages, beginning healing.

terms of mean, standard deviation, coefficient of variation, and their respective probable errors; 38 of the chicks were from groups on diet 634. The remaining 80 received diet 108 and modifications, but failed to show either symptoms or lesions. Since similar results were obtained for all chicks with normal brain, irrespective of diet or sex, data from the different dietary groups were combined.

Within the limits of the experiment, 8 to 60 days, the percentage of

water in the different parts of the brain was found to be constant and independent of the age of the chick or size of the brain.

It is seen that the percentage of moisture of the cerebrum tends to be somewhat higher than that of the cerebellum, and distinctly higher than that of the rest of the brain and spinal cord. The low coefficients of variation indicate that the moisture content of the different parts of the brain of the chick is relatively constant.

Moisture determinations were made on the brains of 36 chicks with nutritional encephalomalacia and these values are also plotted in figure 3. Severe lesions in either cerebrum or cerebellum are seen to be characterized by a definite increase in the moisture content.

The moisture determinations of some of the encephalomalacic chicks are presented in table 1.

Only those parts of the brain which showed pathological lesions had increased moisture content. The mid-brain was histologically normal in every case and the percentage of moisture, determined in 16 cases, was observed to fall within the limits for normal tissue. The increase in moisture occurred in the same tissues as were shown (fig. 2) to have an increased weight. The variations in moisture increase were due to the differences, which have been discussed, in the stage and extent of lesions.

In order to determine the influence of autolysis, 16 normal chicks were chloroformed, and after an interval of from 15 minutes to 8 hours the brain was removed for moisture determination. The increase in moisture was found to occur soon after death. The values obtained, plotted in figure 3, are seen to be higher than normal, but not so high as some of those found in degenerated tissue. In autolyzed brain, both cerebrum and cerebellum apparently attain the same percentage of moisture.

CONCLUSIONS

1. Nutritional encephalomalacia in the chick occurs during the period of active brain growth.

2. The weights of the whole brain, cerebrum, and cerebellum are dependent upon the body weight, and are not influenced by age, sex, or rate of growth.

3. The different parts of the brain reach their maximum weight simultaneously when the chick weighs approximately 550 grams, which corresponds to an age of 9 weeks upon an optimum diet.

4. The percentage of water in the different parts of the brain of chicks, 8 to 60 days of age, was found to be constant and independent of the age of the chick or size of the brain.

5. Acute encephalomalacic lesions in either cerebellum or cerebrum are characterized by increased weight and moisture content.

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CARBOHYDRATE AND ELECTROLYTE CHANGES IN THE OPOSSUM AND MARMOT FOLLOWING ADRENALECTOMY[1]

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Common laboratory animals—cat, dog, rat, rabbit, guinea pig—show diminutions in serum sodium and chloride content following adrenalectomy (Baumann and Kurland, 1927; Silvette and Britton, 1933; 1935; Loeb *et al.*, 1933; Harrop *et al.*, 1933). Addisonian patients who have been observed in crisis are often similarly affected because of adrenal lesions (Loeb, 1932). It has also been demonstrated by the above workers that an increased renal excretion of sodium chloride accompanies the subnormal values found in the blood and tissues after adrenal removal. Adrenal insufficiency may be relieved if compensation for excessive sodium loss is made by the injection or oral administration of sodium salts (Harrop *et al.*, 1935); furthermore, if the sodium content of the body is reduced experimentally, a condition resembling that of adrenal insufficiency is said to result (Gilman, 1934).

Observations on adrenal insufficiency have now been extended to other species besides the commoner laboratory forms. The opossum (*Didelphys virginiana*) and the marmot (*Arctomys monax*) have been particularly studied. Results of experiments on these animals have confirmed the idea that there may be different reactions to adrenalectomy in different animal types.

METHODS. The analytical methods used in the present study have been previously described (Silvette and Britton, 1932, 1933, 1935); other experimental procedures are indicated later. Both male and non-pregnant female opossums were used. Marmots were adrenalectomized and utilized for observation during the spring and summer months, since winter-operated animals may survive many months until the onset of warm weather (Britton, 1930).

RESULTS. *Opossums.* Following one-stage bilateral adrenalectomy the average survival period of 12 opossums was six days (range, 2–10 days). Analysis of the blood serum of animals used when insufficiency symptoms

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

[2] E. R. Squibb and Sons Scientific Fellow in Physiology.

were well defined showed a marked *increase* in sodium and chloride concentrations, in comparison with pre-operative levels in the same animals (table 1-A and 2). Contrasting values found in the opossum and cat are shown graphically herewith (fig. 1). Muscle sodium and chloride were also found to be above the normal levels. Correlated with the rise in serum sodium chloride, there was observed a significant decrease in the

TABLE 1

Sodium, chloride and carbohydrate levels in opossums and marmots before and after adrenal removal

ANIMAL NUMBER	BEFORE OPERATION				SURVIVAL	AFTER OPERATION						
	Serum			Muscle water		Condition at sacrifice	Serum			Muscle water	GLYCOGEN	
	Sodium	Chloride	Sugar				Sodium	Chloride	Sugar		Liver	Muscle
						A. Opossums						
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*per cent*	*days*		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*
1	342	354	100		4	Very weak	357	420	50		0.07	0.20
2	338	343	100		3	In convulsions	354	419	60		0.14	0.18
3	309	393	98		2	Prostrated	366	417	50		0.13	0.21
4	339	379	77	(77.6) *	2	Very weak	380	399	67	(72.7) *	0.10	0.16
5	340	389	98		5	Prostrated	356	432	56		0.16	0.28
6	315	356	77		9	Prostrated	339	393	61		0.17	0.13
7	344	372	82		2	Prostrated	367	411	55		0.18	0.13
8	323	358	90		6	Prostrated	315	360	72		0.08	0.31
						B. Marmots						
1	329	364	171	79.9	1	Prostrated	324	378	50	78.0	0.14	0.31
2	334	353	196	79.1	5	In convulsions	353	406	38	78.6	0.10	0.16
3	336	374	178	79.1	7	Prostrated	329	382	40	77.0	0.09	0.48
4	302	392	107	77.6	28	In convulsions	321	406	48	77.3	0.11	0.43
5	315	374	177	75.1	6	Prostrated	330	380	35	74.5	0.24	0.69
6	302	372	189	76.7	1	In convulsions	339	410	37	76.3	0.21	0.58
7	314	378	175	80.1	4	Prostrated	333	362	63	77.1	0.18	0.50

* Averages taken from table 2.

water content of skeletal muscle. Serum sugar and liver and muscle glycogen figures were reduced to very low values.

In order to determine the effect of adrenal removal on urinary excretion of sodium chloride, normal female opossums were studied individually before and after operation. The animals were weighed and the urinary bladder emptied by catheter; they were then given fluid (see table 3) by

stomach tube and placed in wire-mesh metabolism cages set over granite-ware funnels, the spouts of which dipped into beakers containing a layer of toluene. At the end of each 24-hour period the urine was again withdrawn and the animals weighed. In order to standardize the (adequate) calorific and salt intake, milk was given by mouth on the basis of body weight. In four out of five such continuous metabolism experiments there were notable decreases in chloride excretion after adrenal removal (table 3). Sodium excretion after the operation did not, however, differ

TABLE 2

Sodium, chloride and carbohydrate levels in blood and tissues of normal and adrenalectomized opossums

	SODIUM		CHLORIDE		MUSCLE WATER	SERUM SUGAR	GLYCOGEN	
	Serum	Muscle	Serum	Muscle			Liver	Muscle
Normal								
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*per cent*	*per cent*
Maximum	395	96	426	75	80.2	177	1.01	0.62
Minimum	278	44	343	44	76.1	70	0.33	0.37
Average	336	68	382	56	77.6	100	0.66	0.48
P.E. Average	±4.4	±3.7	±4.1	±2.4		±3.9		
Number of cases	17	9	18	9	8	18	9	9
Adrenalectomized								
Maximum	392	136	457	91	75.1	110	0.18	0.70
Minimum	315	53	360	46	71.4	50	0.07	0.13
Average	360	80	411	60	72.7	69	0.13	0.28
P.E. Average	±3.7	±6.3	±4.9	±3.5		±3.8		
Number of cases	11	6	11	6	5	11	12	12

from the normal. In connection with the latter result it should be remembered that cow's milk contains approximately twice as much chloride as sodium (the milk used contained by test 0.035 per cent sodium and 0.077 per cent chlorides).

Details and results of experiments on five fasting female opossums given 0.9 per cent sodium chloride solution by mouth over three day periods, before and after adrenal excision, are shown in table 4. Compared with the average daily pre-operative excretion, the adrenalectomized opossum excreted only 75 per cent of the administered salt. The results show that

in the absence of the adrenal glands the opossum retains ingested sodium and chloride. Thus the increased serum sodium chloride concentration of the blood serum and tissues of the adrenalectomized opossum appears to be explained.

Marmots. Marmots which had been bilaterally adrenalectomized in a one-stage operation eventually showed characteristic symptoms of insufficiency, as in the case of the opossum and other species. The average survival period in 7 cases was 8 days. When deficiency symptoms had become well developed, the sodium, chloride and water changes were

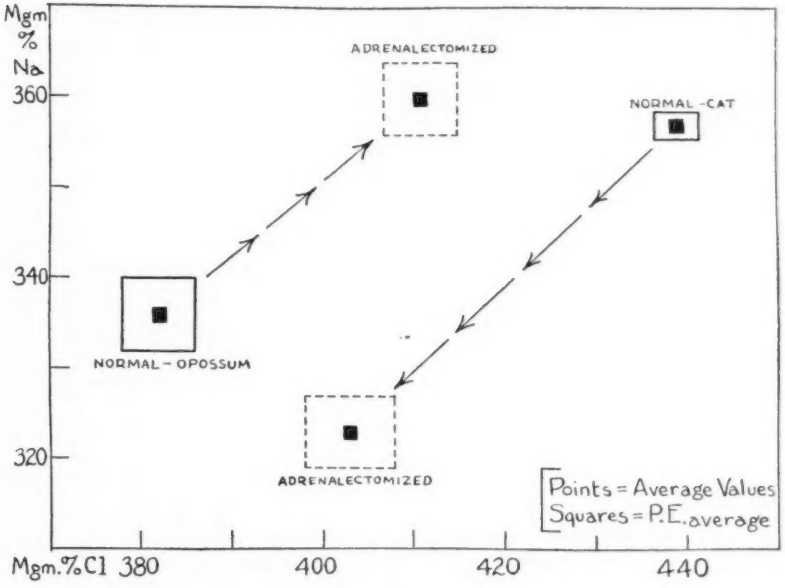

Fig. 1

observed to be similar to those found in the opossum, although not quite so extensive (table 1-B). Both serum sodium and chloride increased about 4 per cent over the normal levels, while increases in the adrenalectomized opossum were 8 and 10 per cent respectively above the normal.

It is to be emphasized that in the experiments on marmots as well as opossums comparison was made on the same animal before and after operation. Serum sugar and liver glycogen in marmots were reduced to very low levels after adrenalectomy, and muscle glycogen also showed a diminution from the normal.

Discussion. For some time past we have considered the possibility

TABLE 3

Urinary output of sodium and chloride in opossums on milk diet before and after adrenalectomy

ANIMAL NUMBER	EXPERIMENTAL CONDITIONS (AMOUNT PER KGM. BODY WEIGHT DAILY BY MOUTH)	OBSERVATION PERIOD BEFORE OPERATION	AVERAGE DAILY URINARY OUTPUT BEFORE OPERATION*			OBSERVATION PERIOD AFTER OPERATION	AVERAGE DAILY URINARY OUTPUT AFTER OPERATION*		
			Water	Sodium	Chloride		Water	Sodium	Chloride
		days	*cc.*	*mgm.*	*mgm.*	*days*	*cc.*	*mgm.*	*mgm.*
11	100 cc. milk	5	30	22	62	9	34	23	40
12	100 cc. milk	5	27	24	67	11	37	20	69
13	100 cc. milk + 50 cc. water	14	82	21	67	2	85	32	57
14	100 cc. milk + 50 cc. water	14	80	25	72	8	83	10	60
15	100 cc. milk + 50 cc. water	8	83	25	68	5	76	34	55

* Per kilo of body weight.

TABLE 4

Normal and post-operative sodium and chloride excretion after saline administration (opossum)

ANIMAL NUMBER	EXPERIMENTAL CONDITIONS†	AVERAGE BODY WEIGHT DURING RUNS	AVERAGE DAILY EXCRETION DURING 3 DAY RUNS*				
			Water	Sodium		Chlorides	
				Urine	Total daily	Urine	Total daily
		kilos	*cc.*	*mgm. per cc.*	*mgm.*	*mgm. per cc.*	*mgm.*
9	Pre-operative	1.75	86	4.03	347	6.83	587
	Post-operative (5–7 days)	1.67	62	4.44	275	7.04	436
10	Pre-operative	1.38	57	5.17	294	8.45	481
	Post-operative (5–7 days)	1.37	73	3.66	267	5.86	427
16	Pre-operative	1.44	90	4.11	369	6.65	598
	Post-operative (5–7 days)	1.43	77	3.86	297	6.39	492
17	Pre-operative	1.55	73	4.58	334	7.10	518
	Post-operative (5–7 days)	1.50	76	3.73	283	6.42	487
	Post-operative (19–21 days)	1.38	72	3.09	222	4.67	336
18	Pre-operative	1.33	74	5.19	383	8.08	596
	Post-operative (4, 8, 14 days)	1.40	34	5.24	178	8.80	299

* Per kilo of body weight.

† One hundred cubic centimeters 0.9 per cent NaCl per kilo body weight daily for 3 days.

of finding an animal species in which two important groups of phenomena which supervene after adrenal removal, *i.e.*, the carbohydrate and sodium chloride changes, might perhaps be separated by Nature—an animal type in which, for example, the carbohydrate levels might become reduced following adrenalectomy while the salt content might remain normal, or *vice versa*. The primary responsibility for the animal's death in such case might directly be indicated. Functional conditions in the opossum and marmot, we have now observed, offer a key to the solution of the problem.

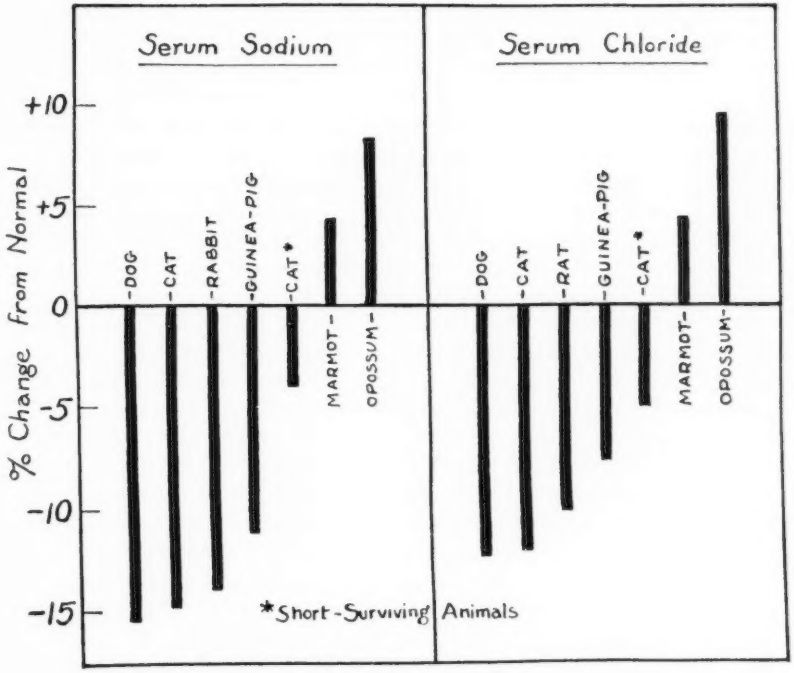

Fig. 2

If such animals are sacrificed when adrenal insufficiency symptoms appear, blood-sugar levels are found to be critically low, and muscle and liver glycogen are profoundly decreased as in the case of commoner laboratory animals. Serum sodium and chloride values, however, show a definite *increase* over the normal controls, which is well maintained until the point of death. Muscle sodium and chlorides are increased, while muscle water percentage is significantly decreased.

Sodium chloride and water balance are therefore strikingly different in

the adrenalectomized opossum and marmot from any other types which have been investigated to date. The graph herewith depicts the sharp contrast in serum sodium and chloride levels in various animals before and after adrenal ablation (fig. 2). Analyses of the daily urinary output of opossums given saline before and after adrenalectomy are shown to be in correlation with the above observations: excretion of sodium chloride is *less* after operation than before.

It is appreciated that the opossum represents one of the lowlier mammalian types, and the marmot also is in many respects an ancient form, perhaps only slightly higher than the opossum (Huber, 1934). Marsupial nursing and hibernating activities, which are noted respectively in these animals, are to be listed among the characteristics of primitive mammals. Nevertheless it is hardly likely that the internal secretory mechanisms of these forms would differ in any qualitative way from those found in the commoner, more highly specialized laboratory animals. Hormones, although not categorical in their expressions, appear to exert their influence under the same conditions with reproducible uniformity through different animal species. Thus, if sodium chloride loss were directly prevented by means of cortico-adrenal hormones in one animal, it would probably be controlled similarly in others.

Changes in renal secretion in adrenal insufficiency with accompanying shifts in salt and water balance, as well as the prolongation of life by saline solutions, may not indicate any specific characteristic of cortico-adrenal function. Sodium chloride and water metabolism may be readily and markedly affected under numerous experimental and pathological conditions (Britton and Silvette, 1936), and saline injections have been shown to extend life in many clinical emergencies. Circulatory disturbances in the adrenalectomized animal—changes in pressure, flow, volume and concentration of the blood—may possibly account for the altered sodium chloride and water balance. It is perhaps unlikely that glomerular filtration would be greatly affected, since permeability of the finer membranes in the body appears to be little disturbed (Barker, Fazikas and Himwich, 1934; Silvette, 1935). The possibility that defective tubular function may be present, however, even in the earlier stages of insufficiency, is seriously to be considered. Interference with reabsorption of sodium chloride and probably of water would thus occur, on the basis of observations by Richards and Walker (1935) that electrolytes are abstracted particularly by the distal portion of the tubules. Such disability in the tubular mechanism may perhaps be explained on the basis of hemodynamic disturbances—in flow, pressure, etc.—particularly in the *vasa efferentia*. Circulatory pathways in the mammalian kidney appear to be arranged primarily to supply the glomerular structures. It seems most likely from anatomical and physiological considerations that tubular func-

tion would suffer first, and perhaps particularly by (functional) destruction of the reabsorptive or filtering ability, in the face of circulatory depression which is known to supervene after adrenal removal. Limitations to tubular reabsorption in other conditions, e.g., glycosuria, are well recognized and possibly comparable. In extensive renal affections, the ability to reabsorb salts may be greatly impaired while sugar may be completely reabsorbed.

Involvement of the pituitary gland in adrenal insufficiency is fairly certain, and may account for some or all of the salt and water changes observed after adrenal removal. Disturbances in water balance and also salt depletion which occur in diabetes insipidus may be recalled in this connection. Experiments on some possible pituitary correlations are now in progress.

In all the animal types which we have studied—cat, dog, rat, guinea pig, opossum, marmot—adrenal removal leads to serious blood-sugar and liver-glycogen depletion. Effects of the cortico-adrenal hormone on carbohydrate metabolism cannot be considered as indirect, or due to inanition, as Harrop (1935) has recently stated. In cats which succumb to adrenal insufficiency in only one or two days after operation the glucose and glycogen levels are found to be markedly reduced, whereas long-term fasting of normal animals (up to 12 days, in which 27 per cent loss in body weight may occur) leads to no diminution even in blood sugar (Silvette, 1934). We are thus still inclined to the belief that defective carbohydrate metabolism represents the most serious effect of lack of the cortical hormone.

SUMMARY

Carbohydrate and electrolyte studies have been carried out in the opossum and marmot, and comparison made with results observed on the commoner laboratory animals (dog, cat, etc.).

In the adrenalectomized opossum and marmot, the sodium and chloride levels in the blood serum and muscle are found to be increased over normal pre-operative values in the same animals. Muscle water is concomitantly decreased.

Daily urinary excretion of sodium and chloride is correlatively subnormal in saline-treated adrenalectomized opossums, compared with the output before operation.

The commoner laboratory forms (dog, cat, rat) which have been examined to date show strikingly opposite changes in salt and water balance following adrenal excision.

It is hardly likely that opposite shifts in sodium and chloride and water balance would be observed after adrenalectomy in different animals if the cortico-adrenal hormone were specifically related to salt and water regulation.

All the mammalian types which have been observed—including the dog, cat, rabbit, guinea pig, rat, opossum and marmot—show unidirectional shifts in carbohydrate values after adrenalectomy: blood glucose and hepatic glycogen are reduced to levels incompatible with life, and muscle glycogen is notably decreased.

The life-maintaining hormone of the adrenal cortex seems to affect sodium chloride balance only indirectly. It appears to be concerned directly in the regulation of carbohydrate metabolism in the organism.

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RELATION OF THE ADRENAL CORTEX TO REPRODUCTION AND LACTATION[1]

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It appears to be a natural function of the mother to supply the fetus with specific chemical factors as well as general nutritive materials throughout the period of pregnancy. The passage of substances in the reverse direction from fetal to maternal organism has also been stated to occur. Clinically the diabetic mother is said to find surcease from her disease, for example, during the gestation period; and laboratory findings also indicate that the occurrence of pregnancy in experimental diabetes results in alleviation of the glycosuria. The preparation of potent extracts of the adrenal cortex has allowed extension of earlier observations on the relation of pregnancy, parturition and lactation to adrenal function. The present paper is concerned with this relationship and includes details of previous preliminary reports (Britton and Kline, 1933, 1935).

RESULTS. *Incidence of pregnancy.* Observations were first made on the incidence of pregnancy following adrenalectomy in two series of white rats. In each of four cages *a*, 16 adrenalectomized females were mated with 4 normal males, and in each of a similar number of cages, *b*, 16 normal females were exposed to four adrenalectomized male animals. Notes on pregnancy were made on these and on a further series of 4 control groups; *i.e.*, 240 animals in all were studied in these experiments. Matings were made within 24 hours after operation. A breeding period of 12 weeks was allowed in each case. Replacement of losses by death was made immediately in the case of adrenalectomized males only.

In the first series *a* 19 of the 64 adrenalectomized females survived through the breeding period, and 5 of these became pregnant and gave birth to normal litters. In 2 cases only was lactation normal, and the litters were reared to weaning. Examination at biopsy or necropsy of those rats which became pregnant disclosed in all cases enlarged accessory cortico-adrenal tissues in the region of the vena cava.

In the second series observed, *b*, 35 of the normal females exposed to adrenalectomized males became pregnant and gave birth to normal litters,

[1] Grateful acknowledgment is made of aid received in this investigation from the National Research Council Committee for Research in Problems of Sex.

most of which were reared. The males which survived in this series showed in all cases small accessory cortico-adrenal tissues.

In normal control series, 44 pregnancies occurred. The fertility of the experimental series *b* was therefore approximately 20 per cent lower than that of the controls.

Adrenalectomy and the course of pregnancy. Pregnancy in the rat is usually not disturbed by even severe degrees of trauma. In numerous cases of thyroidectomy, splenectomy and other procedures performed on the pregnant animal, the processes of gestation, delivery and lactation have followed in a normal manner. These observations made it of interest to determine the effects of partial and complete adrenalectomy—operations which may be carried out on the rat very rapidly (in 2 to 4 minutes) with only a slight amount of trauma.

Removal of one adrenal gland appeared to have an effect on the course of pregnancy in some cases. In 8 experiments, pregnant rats were unilaterally adrenalectomized at different stages of gestation; 5 of these showed normal litters, while in 3 cases resorption of the uterine contents took place. Pregnancy recurred in only one of these 8 animals within the following 12 months.

Bilateral adrenalectomy of the pregnant rat was carried out in a series of 15 experiments. Death of the fetuses usually occurred within 5 days after operation and invariably within 10 days. There was apparently a small amount of fetal growth after operation in a few instances; abortion or resorption of the uterine contents or fetal death *in utero* eventually occurred, however, in all cases. A number of the adrenalectomized rats nevertheless continued to survive for some time. It may be observed that the animals were operated on at different stages of the gestation period.

In a short series (4 cases) in which adrenalectomy was performed on rats which were rearing young, lactation stopped within a few days. All the litters died within one week,—the adult rats succumbed within 3 weeks after operation.

Influence of cortico-adrenal extract on pregnancy and lactation. The administration of cortico-adrenal extract (Britton and Silvette, 1931) to pregnant rats which had been completely adrenalectomized brought about strikingly different results. In 5 experiments (table 1), normal pregnancy, parturition and lactation followed treatment of these operated animals with daily doses of 2 or 3 cc. of extract.

Lactating rats which were adrenalectomized at various times after parturition (3 to 8 days) also continued to nurse and rear their litters normally under the influence of cortico-adrenal extract (table 2).

In most cases, experiments (tables 1 and 2) were not continued for more than 15 to 20 days in order to conserve extract. The animals thus

succumbed after stopping extract, with the usual symptoms of adrenal insufficiency, or they were killed in order to test by autopsy the completeness of the operation. In a few cases the young were reared by adrenalectomized mothers maintained by extract; withdrawal of the injections then

TABLE 1

Results of treatment of bilaterally adrenalectomized pregnant rats with cortico-adrenal extract

RAT	WEIGHT OF RAT	EXTRACT GIVEN	TIME AFTER ADRENALECTOMY YOUNG BORN	YOUNG IN LITTER	RESULTS
no.	*grams*	*cc./day*	*days*	*no.*	
1	121	2	8	4	Normal litter
2	131	2	4	5	4 normal; 1 still-born
3	164	2	1	4	Normal litter
4	122	2	2	5	4 normal; 1 still-born
5	137	3	4	5	Normal litter

TABLE 2

Observations on treatment of adrenalectomized lactating rats with cortico-adrenal extract

RAT	WEIGHT OF RAT	TIME BETWEEN PARTURITION AND OPERATION	YOUNG IN LITTER	EXTRACT GIVEN	RESULTS
no.	*grams*	*days*	*no.*	*cc./day*	
11	126	4	6	3	Young gained weight normally
12	178	4	3	3	Young died 5 days after operation
13	98	4	4	3	Normal growth of young
14	131	4	6	3	Young weaned at age 22 days
15	134	6	5	5	Normal growth of young

resulted in maternal death in one to two weeks. The experiment on animal 9 (table 2) may be quoted in brief:

Rat 9. Litter of 6 young born February 2, 1934. February 6, weight 131 grams; both adrenals removed; 3 cc. cortico-adrenal extract no. 21–24 given daily by intraperitoneal injection after operation. Young continued to be nursed by mother; all gained weight normally. February 14, 2 young placed with another mother with small litter. February 24, 4 young weaned. February 26, extract stopped; weight of mother 138 grams. Symptoms of insufficiency appeared March 8; animal died March 9, weight 121 grams. Young grew up normally.

Partial and complete adrenalectomy and pregnancy in cats. A number of comparative experiments were carried out on cats, particularly because these animals only occasionally (in our experience in 5 to 10 per cent of cases) possess accessory cortico-adrenal tissues. In 20 experiments in

which the adrenals were removed from pregnant cats, abortion (usually within one or two days) or fetal death *in utero* took place. In all cases such adrenalectomized cats died within a few days to two weeks after operation.

Removal of one gland and one-half the other also commonly brought about early abortion, and even unilateral adrenalectomy generally resulted in serious consequences. In both latter types of experiment, however, the operated rat usually survived indefinitely. Brief data are given below.

Group 1: *Both adrenal glands removed.* Results:

Twenty experiments: in 17 cases, abortion of fetuses occurred within 3 days after operation, and often within 24 hours.

In 3 cases, dead fetuses were found *in utero* at death of mother.

All cats died within 3 to 14 days after operation.

Group 2: *Whole of left and half of right adrenal removed.* Results:

Experiment 1—3 half-term fetuses aborted 4 days after operation; cat survived.

Experiment 2—5 near-term fetuses aborted 12 days after operation; cat survived.

Experiment 3—2 kittens born 22 days after operation; died 2 days later; cat showed no lactation, survived.

Experiment 4—2 half-term fetuses aborted 3 days after operation; cat died 4 days later.

Group 3: *Left adrenal only removed.* Results:

Experiment 1—5 half-term fetuses aborted within 3 days; cat survived.

Experiment 2—2 kittens born 2 days after operation, died 2 days later; mother showed no lactation; survived.

Experiment 3—cat aborted; survived.

Experiment 4—cat aborted within 2 days; survived.

Experiment 5—4 half-term fetuses aborted 5 days after operation; cat died 2 days later.

Discussion. Observations which have been reported on the relationship of the adrenals to reproduction and lactation appear in many cases to be fragmentary and indefinite. The early findings of Stewart (1913) that adrenalectomized pregnant or lactating cats survive much longer than non-pregnant animals have not been supported. Pregnant dogs and animals in heat may apparently survive for some weeks after adrenal removal (Rogoff and Stewart, 1927, 1928).

Hartman and his colleagues (1933) state that their adrenal extract, "cortin," is not able to maintain lactation in adrenalectomized rats. Another preparation from the adrenal cortex, "cortilactin," is nevertheless said to be effective. An early report from the Princeton laboratory indicated that the Swingle-Pfiffner extract does not sustain lactation after adrenal removal (Carr, 1931). In a later paper it is stated without experimental proof, however, that adrenalectomized male and female dogs

reproduce normally when kept alive with highly purified Swingle-Pfiffner preparations (Gaunt and Parkins, 1933).

Grollman and Firor (1935) remark that their adrenal "hormones" were unable to repair "dysfunction of the reproductive system" in chronic adrenal insufficiency, but that "a ready response was elicited by injection of extracts from the pituitary." It was concluded that hypophyseal insufficiency is responsible for reproductive failure after adrenal removal, although no protocols or experiments are reported.

SUMMARY

Adrenalectomized female rats seldom become pregnant, and cases in which pregnancy occurs are apparently possible because of enlarged accessory cortico-adrenal tissues. Pregnant animals which have been adrenalectomized fail to go through the normal processes of parturition; abortion is commonly observed, and lactation does not follow. Removal of only one adrenal in the female rat frequently upsets the normal course of pregnancy, and often results in sterility.

In contrast, more severely traumatizing operations than adrenalectomy commonly do not affect the normal course of pregnancy in the rat.

Bilateral adrenalectomy in the cat usually terminated pregnancy within 48 hours. Even partial adrenal removal (of one or one-and-a-half glands) in this animal frequently results in early death of the fetuses.

Adrenalectomized male rats, although in some cases they may survive indefinitely after operation, show reduced fertility compared to normal controls. The presence of accessory adrenal tissues apparently explains the reproductive ability of adrenalectomized males.

Extracts of the adrenal cortex give adequate protection to the adrenalectomized pregnant rat, and allow normal reproduction, parturition and lactation to take place.

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THE VELOCITY OF BLOOD FLOW IN ARTERIES IN ANIMALS

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Most of the methods that have been used for measuring the velocity of blood flow have necessitated sectioning of the vessels, use of anticoagulants and similar considerable interference with the normal state. These disadvantages apply to instruments such as the stromuhr which measures the mean flow, and to those of the type of Broemser's differential manometer (1928), which are adapted to measure velocity changes. To offset some of these undesirable features, Rein (1928) devised his ingenious method in which the blood passing a point is heated by an alternating current passed across the vessel. The rise in temperature thus generated is measured by a pair of thermocouples and varies as the rate of flow. His method is, however, hard to standardize, and cannot follow rapid changes since the thermocouples are not in the blood stream.

The hot wire principle used by A. V. Hill (1920) for the measurement of pulse wave velocity was utilised by Anrep (1927) to measure the flow in the coronary veins by recording the secondary movement of an air column connected to the fluid system. In the present work the hot wire principle is utilized to measure blood flow directly by the insertion of the wire in the moving fluid stream, with a consequent increase in accuracy and ease of standardization and with an enormous increase in the capacity to follow rapid changes without distortion. The application of the hot wire principle to the direct measurement of fluid movement has not previously been made in physiological work; it was applied by Davis (1920) to the measurement of fluid movement in colloid solutions but this earlier work was unknown to the author at the time the method was devised.

METHOD. The hot wire itself consisted of an enamelled nickel wire having a diameter of 63μ and a resistance of 0.285 ohm per cm. at 20.0°C. It was threaded directly through the artery, heated, and its resistance measured, by the use of a Kelvin-Thompson bridge, the diagram of which is shown in figure 1. The bridge was composed of six arms, four of which (*R-I*, *R-II*, *R-III* and *R-IV*) were manganin coils, each of which had a resistance of 100 ohms. The fifth arm (*R-V*) was an adjustable calibrated mercury resistance (*S-W*). It was composed of a column of mercury—102 cm. long and 6.75 mm. in diameter—contained in a glass tube; the

resistance could be changed by altering the thickness of the mercury column. This was done by raising or lowering a solid glass rod 112 cm. long and 6 mm. in diameter, which was plunged into the mercury. The glass tube was mounted vertically in a metal stand. A platinum wire was sealed into the bottom of the tube so as to make contact with the mercury, while the outer end was soldered on to a heavy copper wire leading to the bridge. The upper end of the glass tube had a glass reservoir fused onto it, into which dipped the other lead wire. A metal pointer, fixed to the upper end of the glass rod, slid along a meter stick and indicated the degree to which the glass rod was immersed.

With this mercury column a resistance of 0.144 ohm at 23.5°C. was available. Additional resistance was obtained by connecting the variable resistance in series with a number of manganin coils of various resistances.

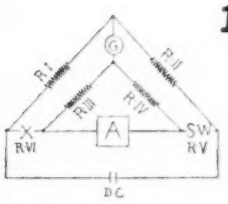

Fig. 1. Kelvin-Thompson bridge. *R-I, II, III* and *IV* are manganin coils of a resistance fixed at 100 ohms each. *R-V* (*SW*) calibrated adjustable mercury resistance. *R-VI*, nickel wire in artery representing unknown resistance. *A*, ammeter. *G*, galvanometer. *DC*, direct current source.

The sixth arm (*R-VI*) was formed by the hot wire already described. A length of 1 to 5 cm. was used; one end, after being freed of its enamelled coating, was soldered to a 4 cm. length of enamelled copper wire (B. & S. no. 26) after which the other end of the nickel wire was threaded through the eyelet of a no. 20 steel needle 5 cm. long. The needle was then pierced through the wall of the blood vessel, threaded through the lumen for the desired length, and then through the opposite wall in such a fashion that the wire entered and left the vessel at opposite poles of 2 diameters parallel to each other. The free end of the wire was drawn tightly to bring the soldered junction on its distal end as close to the external coat of the vessel as possible, and also, if possible, to prevent the wire in the vessel touching the intima at any point except those of entry and exit. The free end of the nickel wire was then bared of its enamel, and a 4 cm. length of no. 29 enamelled copper wire was soldered onto it as close to the adventitia as possible (within 1 mm.), so as not to leave any of the high resistance nickel wire outside the vessel. The edges of the wound were approximated; the two lengths of copper wire protruded through the skin and were connected to the leads running to the bridge.

An ammeter (*A*) indicated the amount of current running in the system; the current was derived from a 6 volt storage battery with appropriate resistances. The electrical balance of the system was indicated by a sensitive D'Arsonval or by a string galvanometer.

The resistance of the wire at minimal current values when its resistance was determined almost entirely by the temperature of the blood stream was first measured. For this purpose a current of 20 milliamperes was found adequate. A direct current of 600 milliamperes was then passed through the system; this amount of current heated the wire in the blood vessel to some 4 to 6°C. above the temperature of the blood stream. The increase in the resistance of the wire thus caused was measured. The resistance of the heated wire now varied according to the changes in flow past it during the various phases of the cardiac cycle; it decreased as it was cooled during systole and increased again as its temperature rose in diastole. The changes in resistance of the wire thus occasioned upset the balance of the bridge and were indicated by the deflections of the galvanometer which were photographed on recording paper. For accurate balancing and determinations of mean flow, the galvanometer used was a Leeds and Northrup, type R; internal resistance of 200 ohms, with a sensitivity of rather less than 1 mm. per micro volt at a distance of 1 meter and a period of 2 seconds; for measurement of rapid changes, a string galvanometer of the Cambridge type was used. It was possible to measure resistances up to 2 ohms; changes in resistance could be estimated with an accuracy of ± 0.0001 ohm.

It is obvious that the current strengths used are only suitable for the velocities commonly found in the arteries investigated. If the velocities are much lower, the wire will be heated to too high a temperature and blood coagulation on the wire will be induced. Experiments on a schema have demonstrated that such lower, or higher, velocities may be accurately measured if the high current is adjusted to give an appropriate rise in temperature, a rise which is indicated at the time of experiment by the percentage increase in resistance. The method is, therefore, perfectly applicable to the measurement of venous velocity, if the current be adjusted appropriately; it has, however, so far not been so applied.

Method of standardization of the wire. The artery containing the wire by which the flow was recorded was divided distally to the wire and a cannula was inserted. To this, other cannulae of different size could be connected by means of rubber tubing so as to permit bleeding from the artery at different velocities. The blood was collected and measured, and the time of bleeding was noted. Resistance measurements were made with both high and low currents during each bleeding. The blood collected after each bleeding was reinjected into the animal in order to maintain its circulating volume; a moderate dose of the anticoagulant Liquoide (Stuher and Long, 1932), namely, 20 mgm. per kgm. of body weight, was injected into the animal previous to the standardization procedure to prevent coagulation of the blood while "in vitro." In some experiments a special cannula was used to allow simultaneous recording of lateral pres-

sure, and in these experiments coagulation was prevented throughout the experiment by the intravenous injection of heparin (80 mgm. per kgm.).

The volume flow in cubic centimeters per second was converted into linear flow in centimeters per second after measurement of the cross sectional area of the vessel. The cross sectional area of the artery at the point containing the wire was determined by connecting a mercury manometer to the artery and pumping mercury into it at a pressure slightly above the pressure in the artery in order to displace the blood in the artery. The pressure in the manometer was then allowed to drop until the mercury pulsated in the vessel at mean pressure, after which a measured length of the vessel was tied off, excised, and the volume of contained mercury weighed. The internal diameter of the vessel could thus be readily calculated.

In those experiments in which it was desired to record lateral blood pressure optically, a T-shaped cannula, containing the wire in the horizontal limb, was introduced into the artery and the vertical limb was attached to a Wiggers manometer. With this cannula, the diameter, and consequently the relation of velocity to volume flow, were known. The standardization values proved to be identical on different occasions, provided that the position of the wire within the cannula was not altered, but slight variations in the position of the wire affected the standardization values.

About four to six bleedings were usually made at different rates of flow. The values thus obtained, when plotted on double logarithmic paper, were found to lie on straight lines.

In figure 2 can be seen four lines representing the results of seven standardizations; three of the seven (15-F, 18-R and 18-L) were the curves obtained with the wire threaded through the carotid or femoral artery directly; the remaining four represent curves obtained with the wire threaded through the special glass cannula. Some of the standardization curves coincided well. Curve 3 (fig. 2) represents three standardizations: 7F, 7FR and 18L. Two of the standardizations, 7F and 7FR, were obtained on the same wire in the same glass cannula, the wire being restandardized in the same vessel at a later time in the course of the experiment. The standardization 18L is that of a wire threaded directly through an artery. Curve 4 represents two standardizations of the same wire in the same cannula in the femoral artery of 2 different animals on different days.

Figure 3 represents a composite of all the standardizations; curve 1 represents the average of nine standardizations with the wire inserted in the vessel directly; and curve 2, the average of eight standardizations with the wire threaded through a glass cannula. From an analysis of all the curves obtained thus far, one factor which causes differences in standardization values appears to be the diameter of the vessel; in a series of curves

which showed the greater percentage changes in resistance, the wire was threaded through vessels of smaller diameter. A probable explanation is that a given length of wire has a greater chance of lying in contact with the intima of a vessel than in the case of a vessel with a larger diameter. In a series of eight curves, in which the wire was threaded through a glass cannula of constant diameter, variations depended on alterations in the position of the wire during cleaning; but none the less, there was less variation than in a series of ten curves obtained when the wire was threaded through vessels of different diameters. The length of wire used did not appear to be a factor in the causation of the different standardization values. Another factor considered was the likelihood of the deposition of fibrin on the wire, since an anticoagulant was not used when the wire was threaded through the blood vessel directly. This possibility was ruled

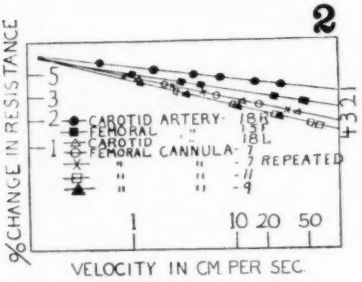

Fig. 2

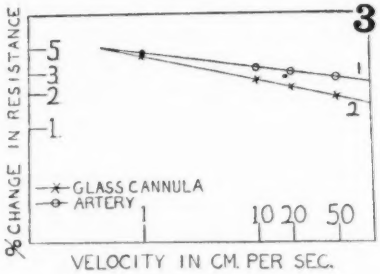

Fig. 3

Fig. 2. Standardization curves obtained in individual experiments plotted on double logarithmic paper. (See text.)

Fig. 3. Average of all standardization curves obtained, plotted on double logarithmic paper. (See text.)

out by observing that the percentage change in resistance for a given wire did not increase throughout the course of an experiment. The longest period during which a wire was allowed to remain in the blood stream was fourteen hours; no clotting or fibrin was detectable on the wire at the end of this time.

Some experiments were also made with a schema and the same type of relationship between flow and resistance change was observed. Inaccuracies were hard to avoid unless the fluid was kept exactly at a constant temperature, as the method is extremely sensitive to changes in the temperature of the fluid. The schema served to demonstrate that variations in the pulsatile flow were unlikely to provide any significant source of error. A semi-intermittent flow had a greater cooling capacity than a continuous flow of the same mean value, but the differences were slight. A blood

stream could be demonstrated in the schema to have a cooling capacity much less than that of a similar stream of water; it is, therefore, probable on both experimental and theoretical grounds that the cooling capacity of a blood stream varies, not only with the specific heat capacity, but also with the viscosity of the blood and may, therefore, vary somewhat in different animals. This provides an additional reason for standardization to be made in each experiment.

In the analysis of records obtained with a string galvanometer, the mean value of the curve was considered to represent the mean velocity and the point of balance with the variable resistance. Deviations from this mean were expressed as changes in resistance by standardizing in each record the excursion of the string for any given change in resistance.

The following data will serve to illustrate the method of calculation. They were obtained in a dog of 13 kgm. weight with a glass cannula of 3 mm. diameter. The length of the wire was 5.35 cm. and it had a resistance of 1.644 ohms at the animal's rectal temperature of 37.5 C. With a current of 600 m.a. the resistance was 1.683 ohms. This represented an increase in percentage change of resistance of 2.33, which, on the glass plunger of the variable resistance, amounted to a shift of 15.5 cm. During standardization, at the end of the experiment, the following values were obtained (curve 4 of fig. 2).

VELOCITY OF BLOOD FLOW	CHANGE IN RESISTANCE	COMMENT
cm. per second	*per cent*	
0 44	5 53	During standardization the high current of 600 m.a. was turned on for the briefest time possible in order to avoid coagulation of blood on the wire at very low velocities
1 24	4 22	
3 60	3 24	
11 42	2 38	
27 90	1 83	

Application of the data obtained throughout the course of the experiment to the standardization curve indicates a mean velocity for this vessel of 12.5 cm. per second, while analysis of the curve obtained indicated a maximum systolic velocity of 38.5 cm. per second and a minimum diastolic velocity of 9.2 cm. per second. Calculation indicated that the mean temperature of the wire in the blood stream with the high current was 45.8 C.

Preparation of the animal. The animals used were dogs, anesthetized with dial 0.25 cc. per kgm., after a preliminary dose of 4 mgm. of morphine sulphate per kgm. An electric heating pad was used to maintain body temperature. Most of the records were obtained in the carotid and femoral arteries which were alone used for the standardization experiments. In some of the experiments the flow was measured in more than one vessel. Readings were taken at short intervals in the various vessels in order to make comparable readings under the same conditions of the animal.

When the carotids were used, care was taken not to carry the dissection nearer than 2 cm. from the bifurcation in order not to interfere with the carotid sinus. The femoral artery was exposed throughout its length in

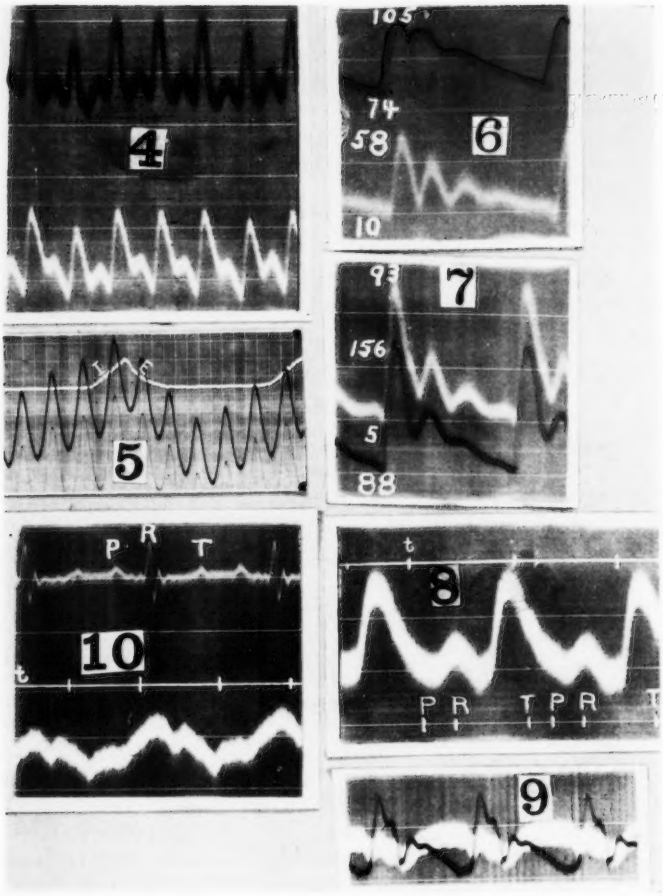

Fig. 4. Simultaneous recording of lateral blood pressure (upper line) and blood flow (lower line) in the femoral artery, using a string galvanometer. The wire was threaded through the horizontal arm of a cannula attached to an optical recording manometer. A complete respiratory wave is shown.

Fig. 5. Respiratory wave in blood flow in the femoral artery using mean flow galvanometer. White line represents respiration with phases as depicted. Dark line represents mean blood flow with wire threaded through femoral artery directly.

Fig. 6. Comparison of velocity and pressure curves at same point in carotid artery. Upper curve, lateral blood pressure with values in millimeter mercury as recorded by optical recording manometer. Lower curve, blood flow with values in centimeters per second as recorded by string galvanometer. Wire threaded through glass cannula. (Blood pressure record retouched.)

Fig. 7. Comparison of velocity and pulse pressure curves at the same point in

Scarpa's triangle; its branches were preserved in all cases except those in which the artery was cannulated for the purposes of recording blood pressures simultaneously. The jugular vein was cannulated to enable the restoration of blood withdrawn from the animal during the standardization.

RESULTS. Examples of curves obtained which show the general characteristics of the velocity changes are shown in figures 4 to 10, and the actual values are indicated in tables 1 and 2.

1. *Shape of velocity pulse curves.* a. *Femoral and carotid.* Simultaneous recording of blood pressure and blood flow at the same point in a vessel, using a wire threaded through a lateral pressure cannula, revealed that the shape of the velocity curves in the femoral artery was very similar to that of the pressure curves, and the resemblance persisted throughout a respiratory cycle (fig. 4). The respiratory variations in blood flow as demonstrated by a slow sensitive mean flow galvanometer can be seen in figure 5.

The relative shapes of the velocity curves in the carotid and femoral arteries of the same animal (figs. 6 and 7) were found to be the same as those generally accepted for blood pressure. The curve in the carotid was of a low broad type, that in the femoral was more peak-like. The velocity and pressure changes occurred at approximately the same time but the start of the increase in velocity and its peak appeared to precede slightly the corresponding pressure changes, as they should on theoretical grounds (confirming the work of Broemser, 1928).

b. *Aorta.* In figure 8 can be seen the shape of the velocity curve in the root of the aorta. The wire in this case was threaded transversely across the aorta about 2 cm. above the level of the aortic valves. A simultaneous recording of the electrical variations (lead II) was used to time the various

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the femoral artery of the same animal as in figure 6. Upper line, blood flow with systolic and diastolic values in centimeter per second, as recorded by a string galvanometer. Lower line, lateral blood pressure with systolic and diastolic values in millimeter mercury as recorded by an optical recording manometer. Wire threaded through glass cannula.

Fig. 8. Velocity curve in the root of the aorta with wire threaded transversely across the vessel about 2 cm. above the level of the aortic valves. Time in 0.2 second (t). Upper line. Middle line, blood flow. Lower line, electro-cardiograph tracing, lead II, indicated but not shown; P, beginning of P wave; R, beginning of R wave; T, end of T wave.

Fig. 9. Velocity curve in anterior descending branch of the left coronary artery (white line) using string galvanometer. Dark line represents simultaneous optical record of end pressure in the left carotid artery. An upstroke indicates an increase in flow in this, as in all the other figures. It may be noted that the flow commences to increase at the end of the systole and reaches its maximum at the start of systole.

Fig. 10. Simultaneous record of electro-cardiographic tracing (upper line, lead 2) and velocity flow in the anterior descending branch of the left coronary artery lower) line) using a string galvanometer. t, time in 0.2 second.

phases of the cardiac cycle; for this a Salamonson galvanometer was used, arranged in tandem with the other galvanometer. The end of systole was indicated by a flattening of the wave, contemporaneous with the end of the T wave; auricular contraction gave a definite change in velocity, while the isometric contraction gave only a slight wave. The fact that the velocity does not fall to zero in diastole indicates that the distensi-

TABLE 1

Values for velocity of blood flow in centimeters per second in 7 carotid and 7 femoral arteries of 9 different dogs

		CAROTID	FEMORAL
Systolic	Average	37.0	33.3
	Maximum	58.0	93.0
	Minimum	13.0	6.2
Mean	Average	12.4	7.8
	Maximum	17.5	13.5
	Minimum	7.2	3.6
Diastolic	Average	9.7	5.0
	Maximum	15.0	9.6
	Minimum	6.0	1.2

TABLE 2

Comparisons of velocity values in centimeters per second of comparable pulse cycles in comparable respiratory waves (not identical) in the carotid and femoral arteries of the same animals

Lateral blood pressure values of the respective cycles are also given.

DOG	VESSEL	BLOOD FLOW			LATERAL BLOOD PRESSURE		
		Systolic	Mean	Diastolic	Systolic	Mean	Diastolic
9	Carotid	49.0	14.0	10.0	104	95	90
	Femoral	52.0	12.5	8.2	129	95	83
11	Carotid	58.0	14.0	10.0	105	81	74
	Femoral	93.0	9.0	5.0	156	97	88

bility of the aorta in the 2 cm. between the valves and the wire, and possibly of the valves themselves, is considerable.

c. *Coronary artery.* The shape of the pulse curve in the anterior descending branch of the left coronary (chest closed) using the string galvanometer can be seen in figures 9 and 10.

Simultaneous recording of end pressure in the left carotid by means of a Wiggers manometer showed that the coronary inflow was decreased during cardiac systole and increased during cardiac diastole. The flow was least

at the end of systole. The significance of the negative wave which occurred early in systole is not clear (fig. 9). Records obtained in another experiment, figure 10, with a simultaneous electrocardiograph, indicated that the wave occurred shortly after the S wave, so that if it is due to the isometric contraction the effect is apparently produced after some lag. The results obtained here confirm those obtained by Anrep (1927) and by Wiggers (1933), by the use of perfusion methods. They do not confirm those of Hochrein (1931) who used the Rein thermo-stromuhr, and who found that the moments of maximal flow varied widely with a tendency to be maximal during systole and minimal during diastole.

2. *The magnitude of velocities in the femoral and carotid arteries.* The values obtained for linear velocity in the femoral and carotid arteries are tabulated in tables 1 and 2.

In table 1 there can be seen values for linear flow in centimeter per second, obtained in 7 carotid and 7 femoral arteries of 9 consecutive dogs. They represent average, maximum and minimum values for systolic, mean and diastolic flow under all sorts of experimental conditions, e.g., initial stages of shock, varying degrees of anesthesia, slight blood loss, temperature, etc.—though all were obtained under conditions that would be usually classed as physiologically normal. It is important to recognize that in anesthetized animals both velocities and volume flow in large arteries may be reduced much below normal values and yet the animal may not appear in any way abnormal by ordinary methods of examination. In this series of consecutive experiments a comparison of values indicates a higher average systolic, mean and diastolic flow in the carotid than in the femoral. However, from the relative shapes of the velocity curves in the two vessels (figs. 6 and 7), one would expect that in the same animal the systolic flow in the femoral would be higher than that in the carotid. That this is the case can be seen in table 2, in which are tabulated the values obtained in two animals in which the flow and lateral pressures were recorded in the carotid and femoral arteries of each. In both of the animals the systolic flow was higher in the femoral, while the mean and diastolic flows had the same relationship as in table 1. The values are taken from pulse curves taken at the same point in comparable, but not in identical, respiratory waves.

Discussion. In the comparison of the relative velocities in the carotid and femoral arteries, the values given in table 2 are more reliable than those of table 1, in that the data were obtained in the same animals under similar conditions. Since it is easier to cause subnormal velocities than hypernormal, it would appear that the higher values should receive the greater emphasis. Systolic velocities of 50 to 100 cm. per second must be considered as normal for animals under anesthesia of the type used.

The data of both tables indicate that the mean velocity was less in the

femoral than in the carotid under the conditions of these experiments, and those of table 2 indicate that, under comparable conditions, the diastolic velocity is lower in the femoral artery. In spite of these differences in favor of the carotid, the change in contour of the femoral curve may result in a systolic velocity which considerably exceeds that in the carotid, and, under such conditions, the pressures in the two vessels may also differ considerably. Estimates of the velocity of blood flow from the size of a vessel and the volume flow are adequate for mean velocity, but are quite incapable of indicating the order of magnitude of systolic velocities which may be 2.5 or 10 times the mean value.

The values for mean velocity can also be expressed in terms of volume flow as far as the times immediately preceding standardization are concerned; in fact, the actual standardizations were primarily in this form. On this basis the volume of normal blood flow at the end of an experiment varied in the carotid from 34 cc. per minute in a dog of 13.7 kgm. to one of 110 cc. per minute in a dog of 17.2 kgm.; the femoral flow similarly fell between the extremes of 11 cc. per minute in a dog of 17 kgm. and 57 cc. per minute in one of 24.8 kgm. However, it is probably not warranted to apply such standardization values to the determination of volume flow at some other stage of the experiment, for any such application implies that the diameter of the vessel does not alter. Such an assumption is unwarranted; in fact, both casual observation and measurement of the external diameter of the vessels definitely indicated, even on the larger arteries, that the diameter varied considerably during the course of an experiment and was usually greater at the start of an experiment than later. The contrast between this method and that of Rein should be kept in mind as they each have their special value. In this method velocity is measured and may be transposed to volume flow if the diameter of the vessel is known; volume flow may always be deduced if a glass cannula containing a wire is inserted; in Rein's method, volume flow is measured and velocities can only be deduced if the diameter of the vessel is known.

SUMMARY

1. The velocity of blood flow in arteries may be measured by the insertion in the artery of a short length of nickel wire. If this be connected to a Kelvin-Thompson bridge and be used according to the hot-wire principle developed by A. V. Hill, the velocity of blood flow may be measured by variations in resistance. With a current of low amperage the resistance is first measured at the temperature of the blood; the current is then increased to a higher value (600 m.a.) such that the temperature of the wire is raised some 4 to 6°C. above the temperature of the blood, and the percentage change of resistance is determined. This increase in

resistance depends upon the change of temperature which varies inversely with the velocity of blood flow. Owing to the small mass of the wire used (63μ diameter) and the intimate contact with the moving stream, the system is able to follow accurately rapid changes in velocity. The method provides, therefore, a means of measuring these changes with little interference with the vascular system. It measures velocity of flow rather than volume flow, though the latter is estimated if the diameter of the vessel is known, or if the wire is contained in a glass cannula of known diameter. The method of standardization "in vivo" is described.

2. The velocity curves of the carotid and femoral arteries are shown to resemble the pressure curves in these arteries and to differ from one another just as do the pressure curves. The mean and diastolic velocities were lower in the femoral than in the carotid, yet, in spite of this, the peak-like character of the femoral pulse usually caused a systolic velocity that was higher in the femoral artery. The differences between the carotid and femoral pulses depend mainly on the variations in the distribution of energy in time.

3. When an animal is in good condition, the systolic velocities observed in the carotid and femoral arteries under dial-morphine anesthesia are usually 50 cm. per second or more. The highest value recorded was 93 cm. per second in the femoral artery. The mean velocities observed were much lower (9 cm. per second). The mean velocities which are calculable from volume flow and the diameter of the vessel, and the recorded maximum systolic velocities are values of quite different orders of magnitude.

4. Records of velocity obtained from the aorta, 2 cm. distant from the valves are shown. The flow at this point does not decrease to zero during diastole; the reservoir action of the aorta and of the aortic valves proximal to this point must, therefore, be considerable.

5. Records of velocity changes in coronary arteries demonstrate that the main flow is in diastole. Some flow is obtained in systole, but the minimum flow is observed toward the end of systole.

The author wishes to express his indebtedness and gratitude to Dr. H. C. Bazett, who suggested and directed the application of the hot wire principle of A. V. Hill to the study of blood flow in the vessels of experimental animals.

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THE EFFECT OF OVARIAN HORMONE ON THE BASAL METABOLISM OF EXPERIMENTAL HYPERTHYROID RATS

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Following the investigation of Sherwood, Savage and Hall (1933) data have accumulated which show that the primary sex hormone has an effect on the experimental hyperthyroid rat as well as the otherwise normal ovariectomized animal.

Reiss and Pereny (1928) were able to raise the threshold of response to oestrin when thyroid was given to castrate rats. VanHorn (1933) reported that three rat units of theelin were necessary to produce oestrus in experimental hyperthyroid rats. He further demonstrated the relation of the thyroid to the ovaries by way of the hypophyseal experimentation. Starr and Patton (1934) were able to produce a decided decrease in the basal metabolism of a hyperthyroid individual by the administration of theelin and antuitrin S.

Since many conflicting results have been reported on the effect of ovarian hormone on basal metabolism and many attempts have been made to show the relationship existing between the thyroid, hypophysis and ovarian function, the present investigation was undertaken.

EXPERIMENTAL PROCEDURE. The experimental procedure was designed to determine the effect of ovarian hormone on castrate and experimental hyperthyroid albino rats. Basal metabolism tests were made in the attempt to show a possible relationship between the thyroid and ovarian functions. The procedure was also designed to check previous reports by Laqueur, Hart and DeJongh (1926), McClendon and Burr (1929), Sherwood, Savage and Hall (1933).

Twenty-six adult albino rats were used in this work. The environmental temperature as well as that of the metabolism room was kept constantly at 28°C. The animals were fed a well balanced grain ration which was supplemented with fresh vegetables, milk, bread and meat. This diet was kept constant throughout the experiment.

The apparatus used was a modification of the Haldane open circuit type (1892). The individual tests were run three hours, a procedure re-

ported successful by Sherwood, Savage and Hall (1933). Diack's formula (1930) for the determination of the surface area was used.

The animals were fasted eighteen hours prior to the test. Experiments were made between 10 a.m. and 4 p.m. for the purpose of eliminating diurnal variations as reported by Horst, Mendel and Benedict (1934). Series of control metabolism tests were obtained before and after ovariectomy for the purpose of determining a possible change due to the operation.

Seven animals were used as theelin controls. Each animal was injected daily for a period of 2 to 8 days. Four rats were injected daily with amniotin for a period of 3 or 4 days. Six rats were used as controls for the potency of the desiccated thyroid. These rats were given from 500

TABLE 1

Effect of ovarian hormone on basal metabolism

RAT NUMBER	WEIGHT	PER CENT DECREASE	DAYS THERAPY	TOTAL RAT UNITS
		Theelin control		
	grams			
1	167	*	7	300
2	180	*	8	120
3	165	10	2	100
4	285	12	7	575
5	308	18	7	575
6	210	21	3	45
7	150	24	2	100
		Amniotin control		
8	285	28	4	275
9	285	30	4	275
10	330	38	4	275
11	300	54	3	200

* Animals 1 and 2 did not show a decrease in basal metabolism.

to 1000 milligrams of desiccated thyroid per kilogram of body weight per day for a period of 3 to 9 days. Nine experimental animals were used to show the effect of ovarian hormone on hyperthyroidism. Each animal was fed thyroid for a period of 3 to 9 days. This was immediately followed with amniotin injections for three days.

The amniotin (Squibb) was injected in an oil solution and the theelin (Parke, Davis) in a water solution. The desiccated thyroid (Parke, Davis) was used in powdered form.

RESULTS. Table 1 represents the data obtained on the product controls, namely, theelin and amniotin. It is demonstrated that theelin caused a decrease of only 10 to 24 per cent in the basal metabolism. Two

rats showed no decrease in heat production. However, the amniotin produced a decrease of 28 to 54 per cent in the basal metabolism.

TABLE 2

Effect of ovarian hormone on experimental hyperthyroid rats

RAT NUMBER	WEIGHT	BASAL METABOLISM (PER CENT RISE)	DAYS THERAPY	THYROID PER KILO (MILLIGRAMS DAILY)	TOTAL RAT UNITS	RETURN TO NORMAL BASAL METABOLISM
Thyroid control						
	grams					*days*
12	200	73	9	500		10
13	245	75	3	1,000		10
14	230	78	3	1,000		9
15	185	62	3	1,000		8
16	205	75	3	1,000		8
17	240	66	3	1,000		8
Experimental						
18	160	106	9 3	500	300	5
19	215	63	9 3	500	300	5
20	265	63	3 3	1,000	300	4
21	280	65	3 3	1,000	300	3
22	185	66	3 3	1,000	300	3
23	200	66	3 3	1,000	300	4
24	155	70	3 3	1,000	300	4
25	145	72	3 3	1,000	300	4
26	220	63	3 3	1,000	300	4

Table 2 shows an increase of 62 to 78 per cent in heat production of the thyroid controls. The basal metabolism returned to normal in from 8 to 10 days. In the experimental animals an increase of 63 to 106 per

cent in heat production was obtained. The experimental animals' basal metabolism was returned to normal in 3 to 5 days when injected with amniotin.

Protocols I and II are typical of the data obtained in the present investigation. Protocol I shows a maximum caloric output 48 hours following the cessation of thyroid feeding and a return to normal eight days after thyroid feeding. Protocol II shows a maximum heat production 48 hours after thyroid feeding and a return to the normal basal metabolism three days after thyroid feeding.

Protocol I. Rat 16. Adult female. Ovariectomized

DATE	WEIGHT	R.Q.	CALORIES KILOGRAM HOUR	CALORIES SQ. M. HOUR	REMARKS
	grams				
January 8	208	0.76	4.15	32.9	Normal
January 17	200	0.73	4.19	32.8	Normal
January 20	208	0.75	4.06	32.1	Normal
January 22	205	0.75	4.27	33.7	Normal
January 24	219	0.77	4.33	34.9	Normal
January 27					Desiccated thyroid, 1000/mgm./kilo
January 28	205	0.72	5.68	44.8	Desiccated thyroid, 1000/mgm./kilo
January 29	201	0.73	6.39	50.1	Desiccated thyroid, 1000/mgm./kilo
January 30	198	0.76	7.13	55.7	
February 1	197	0.73	7.85	61.2	Maximum
February 2	200	0.76	6.89	53.9	
February 3	210	0.75	6.14	48.9	
February 5	206	0.76	6.48	51.1	
February 6	206	0.74	5.52	43.7	
February 7	212	0.76	4.07	32.5	Normal
February 8	208	0.72	4.33	34.3	Normal
February 10	197	0.73	4.50	33.9	Normal
February 11	201	0.70	4.64	36.4	Normal

Discussion. It is definitely demonstrated that theelin and amniotin have an effect on basal metabolism. In the case of theelin as well as with amniotin injections, the initial decrease in heat production of normal animals is followed by a slight rise above the normal level which is apparently an over-compensation following an inhibition of the thyroid gland function. The rise in basal metabolism reported by Laqueur, Hart and DeJongh (1926), McClendon and Burr (1929) corresponds with the secondary rise found in the present investigation. The type of reaction obtained could be explained by the fact that the ovarian hormone might have caused an inhibition of the thyroid function by way of the anterior lobe of the hypophysis.

Excessive doses of theelin and amniotin were administered. This is clearly demonstrated in some of the animals where fewer rat units of either hormone showed the same or a greater effect than in those animals which had received far greater amounts of the hormone. It is realized that the dosage for the present experiment could have been better standardized upon ovariectomized and thyroidectomized animals.

Since considerable time is necessary in metabolism tests of this type, a slow absorption of the hormone is an advantage, as was demonstrated in the present investigation by the use of amniotin in an oil solution. The water solution of theelin did not show as pronounced an effect as amniotin

Protocol II. Rat 22. Adult female. Ovariectomized

DATE	WEIGHT	R.Q.	CALORIES KILOGRAM HOUR	CALORIES SQ. M. HOUR	REMARKS
	grams				
January 18	194	0.77	5.75	44.6	Normal
January 20	185	0.76	5.30	41.0	Normal
February 1	187	0.74	5.20	39.8	Normal
February 2	194	0.75	5.19	40.4	Normal
February 2					Desiccated thyroid, 1000/mgm./kilo
February 3	190	0.75	6.04	38.8	Desiccated thyroid, 1000/mgm./kilo
February 4	192	0.80	6.64	51.1	Desiccated thyroid, 1000/mgm./kilo
February 5	185	0.78	7.84	59.8	Amniotin, 100 rat units
February 6	182	0.77	8.77	66.6	Amniotin, 100 rat units
February 7	183	0.77	5.54	42.1	Amniotin, 100 rat units
February 7					Normal
February 8	185	0.74	6.07	44.9	Normal
February 9	187	0.74	5.56	42.6	Normal
February 10	189	0.77	5.55	42.6	Normal
February 12	196	0.71	5.02	39.0	Normal

when injected as many as four to eight times in 24 hours. With the type of control used, a change in testing time was not suitable for the normal procedure. It is therefore possible that the greatest effect of theelin was not obtained.

Since theelin caused such a slight decrease in the basal metabolism of normal animals, it was decided not to use this product for the reduction of metabolism in the experimental hyperthyroid rats.

The work of Starr and Patton (1934) as well as the consistent data of the present investigation indicates an influence of the ovarian over the thyroid function in the control of basal metabolism.

SUMMARY

The report of Sherwood, Savage and Hall (1933) has been confirmed, namely, that ovarian hormone injections cause a decrease in the basal metabolism of rats.

The ovarian hormone injected as an oil solution produces a greater measurable effect on basal metabolism than does a water solution.

When the basal metabolism was increased as much as 106 per cent by thyroid feeding, amniotin injections brought about a return to normal in less than one-half the time necessary for the usual return to normal in the basal metabolism of the hyperthyroid rat.

We have pleasure in expressing our thanks to Parke, Davis and Company for their generous supply of theelin and desiccated thyroid; to E. R. Squibb and Sons for their amniotin; to Doctor and Mrs. M. M. White for their helpful criticism of the manuscript.

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CONTROL BASAL DIETS IN ANEMIC DOGS

Method Factors and Hemoglobin Production

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These standard dogs are continuously anemic at a level of about $\frac{1}{3}$ normal hemoglobin from about 1 year of age to old age and death—often a period of over 10 years. Efforts are made to exclude all possible variables from their environment and to this end they are protected against infection, kept in air-conditioned rooms at a relatively constant temperature, kept clean in individual cages, and under constant observation. We have studied various basal rations but usually feed the standard salmon bread. It is shown in table 1 below that *method of preparation* of this bread is a factor and machine mixed bread apparently is more digestible and produces more hemoglobin per week. We have used longer basal bread periods (10 to 12 weeks) to get accurate average base line figures and we test the dogs more frequently with standard amounts of pig liver and optimum doses of iron by mouth. This accurate standardization is of considerable importance when we use amino acids or other untested protein fractions added to the basal ration as we should know within a few grams of hemoglobin the expected output on the basal ration alone.

In spite of every effort toward standardization there are at times unexplained reactions which differ from the assumed base line reaction—we call them unexplained physiological variables and for this reason always repeat experiments over and over again in different dogs. There are considerable differences between different dogs, even among litter mates, but this is not surprising. It is well known that some animals can run faster or farther than others and it need not surprise one to observe that one dog can produce a good deal more new hemoglobin than another (4). For this reason one must test any given material on a number of dogs to get a figure which will represent the average potency of a given food substance. Gradually in an anemia colony certain dogs become established as having a *subnormal capacity* to form new hemoglobin in anemia—others can produce much *more than the average* amounts of hemoglobin when given the standard food factors like salmon bread, liver or iron.

Methods are changed slightly from time to time in any long continued

experimental program and it seemed proper at this time to describe the present experimental procedure exactly as it is carried out in this laboratory. Other investigators (2, 7) are beginning to use this type of animal to study the effect of various factors on hemoglobin production and if comparisons are to be made we should know all details of the experimental routine.

METHOD. *Experimental animals.* Dogs which were born and raised in our own kennels are used in all experiments. A small number of white bull terriers and coach terrier mongrels are reserved for breeding purposes. Shortly before the birth of puppies the mother is isolated on the upper floor of the animal building in a room containing several large pens. The first vaccination against distemper occurs when the pups are 7 weeks old and one-half of the normal dose is given. When 3 months old a full dose of vaccine (Laidlaw-Dunkin) is administered. Fecal examinations for parasite eggs are routine and suitable anthelmintics are given when required. At the age of about 6 months the pups are placed in single cages in the anemia colony quarters (9). At about one year of age they have attained their adult weight and are then added to the anemia colony. The dog numbers may seem needlessly cumbersome but the first two figures give the year in which the dog was taken into the anemia colony and reduced to the anemia level. The other figures relate to the serial dog records of the animal house. The same number is used throughout the life of the dog and it is a simple matter to follow any given dog through many papers and tables which have been published. At all times the dogs are kept properly isolated from infection and are handled by a caretaker who does not come in contact with other animals. Before these dogs are rendered anemic they are trained in order to accustom them to technical procedures of the anemia program. For about a week they are placed daily on the operating tables and repeated hypodermic venous punctures are made. They are also taught to take a stomach tube. After this preliminary training period the dog will be undisturbed by withdrawal of a considerable volume of blood and there is no danger of struggling and needle withdrawal with subsequent hematomas. Great care is taken to keep the sites of venous punctures free from skin irritation. A salve made of equal parts of powdered sulphur and vaseline or an iodine containing ointment is useful. The neck is carefully cleaned and ointment thoroughly rubbed in following each venous puncture. By means of this technique we have been able to withdraw blood by hypodermic needle from jugular veins several times each week over periods up to ten years or more without venous thrombosis or fibrosis.

The *normal hemoglobin levels* for these dogs range from 130 to 160 per cent (13.8 grams hemoglobin = 100 per cent) or 18 to 23 grams hemoglobin per 100 cc. blood. We reduce these dogs from the normal level to the standard

anemia level of 45 to 50 per cent hemoglobin by frequent bleedings usually lasting over a period of 50 to 60 days (6). This anemia level of 45 to 50 per cent is maintained as constant as possible by frequent samplings and blood withdrawal to remove the surplus of new hemoglobin. Blood volume determinations are made weekly during this depletion period and are of distinct value as a guide to fix the amount of each bleeding. Also during this depletion a 10 per cent glucose solution is usually given intravenously after the blood withdrawal and in equivalent volume to lessen the chance of shock and clinical disturbance. It is known that these dogs have a reserve store of hemoglobin building materials in the body (6) and by this means this reserve is exhausted and the subsequent reactions to standard diet factors become uniform, as reported in other publications (8).

When a potent diet factor like liver is added to the basal ration we note usually on the third day subsequently a rise in hemoglobin production which attains a high level by the end of the first week's feeding and this output of hemoglobin is sustained during the second week of liver feeding. The liver supplement is then discontinued but the hemoglobin output remains high in the following basal bread week and there is usually some "carry over" into the 2nd and 3rd after-weeks. This obviously means that during this favorable period (liver) the anemic dog stores hemoglobin producing factors which are called out in the subsequent 2 or 3 weeks of basal bread ration. By the 3rd or 4th week of the after period the dog is back to its standard basal hemoglobin output. For the sake of accuracy we have found it desirable to give each dog a 10 week period on standard salmon bread to establish this basal output and it is well to repeat this test every year or two.

Net hemoglobin production means the output of new hemoglobin which is attributable to the supplemental food factor and it is calculated as follows. The gross hemoglobin output for any given period includes all blood withdrawn, even the small samples taken for hematocrit estimations. From this gross total we subtract the hemoglobin which is due to the basal bread ration as determined by long periods on the basal diet alone. Finally we make an adjustment for the difference in hemoglobin level in the dog at the start and at the end of each experimental period—usually a five week interval of which 2 weeks cover the feeding period and 3 weeks include the after period during which any "carry over" is exhausted. A ten kilo dog will have in its circulation about 55 grams hemoglobin at an anemia level of 50 per cent. We therefore allow 1 gram of hemoglobin for each per cent difference in hemoglobin level and know by experience that this is a close approximation. Let us say that a dog shows a 50 per cent hemoglobin level at the start of the experimental period and during the after period it is bled a little too much giving a 44 per cent hemoglobin level at the end of the experimental period. Obviously this 6 grams hemoglobin is not due to

hemoglobin production but to over bleeding and is therefore deducted from the gross total hemoglobin to give the net hemoglobin production.

The *anemia history* of the dog is continuous from year to year and the various periods tabulated are taken from these continuous histories. Unless note is made to the contrary the dog is clinically normal and active.

Blood volume. The dye employed for this purpose is brilliant vital red (Evans-Schultz 370) manufactured by the National Aniline and Chemical Company of New York. When a new lot of dye is purchased it is carefully tested as to toxicity. A curve of dye elimination from the blood stream is also determined. We use a 1.5 per cent aqueous solution and this is made up in 600 cc. quantity. To insure proper solution and sterility it is brought to a boil. After cooling the dye solution is filtered and kept in a cabinet. From this larger quantity sufficient solution for one week's use is taken. This prevents deterioration and fading of the dye. If a precipitate has settled out the material is again filtered before using. With these precautions we have had no unfavorable symptoms with blood volume determinations.

For blood volume determinations a special hypodermic needle $2\frac{1}{2}$ inches long, gauge 18, is inserted in a jugular vein and 10 cc. of blood withdrawn into a dry vaselined syringe. The blood is immediately transferred to an accurately calibrated graduated 15 cc. hematocrit tube containing 2 cc. of a 1.4 per cent solution of sodium oxalate (isotonic for anemic dog blood). The blood and oxalated solution are carefully mixed by inversion and the tube is stoppered. Immediately after withdrawing the 10 cc. sample, the needle is kept in situ and a few drops of blood are permitted to escape. Both red cell and leucocyte diluting pipettes are then filled. The previously measured amount of dye is drawn up into another syringe using 10 cc. of 0.9 per cent salt solution for washing of the dye container. The dye is now injected slowly and the syringe is washed free of dye by withdrawing and reinjecting a few cubic centimeters of blood. The amount of dye used for this purpose permits of some variation within a reasonable limit since there may be individual taste in color readings. Our method calls for 1 cc. per 5 kgm. of body weight. After injection of dye the needle is withdrawn. Four minutes later a clean needle and syringe are used for the second 10 cc. sample obtained from the jugular vein on the other side and placed into an hematocrit tube with oxalate solution. The hematocrit tubes are centrifugalized at 2500 revolutions a minute for 35 minutes. The centrifuge head measures 40 cm. in diameter between sockets for tube holders. The total contents of the tubes as well as of both total cells and red cells are noted. Three cubic centimeters of the dye-colored plasma are withdrawn and diluted in a tube containing 6 cc. of 0.9 per cent salt solution. The unknown is then compared in a colorimeter against a standard. Plasma and blood volumes are estimated according to the formula previously published (1).

Red cell hematocrit values are determined from the sample of blood withdrawn before dye injection. Centrifugalization for 35 minutes gives maximal packing. Actual red cell percentage is estimated rather than total cell volume which would include leucocytes and platelets as well.

Red cell and leucocyte counts are made by the familiar technique from blood taken from the jugular vein direct prior to dye injection for blood volume determination. One hundred squares are counted and the actual figures in each square are tabulated with the adding machine. Counting pipettes are shaken for 3 minutes by a mechanical device.

Hemoglobin pigment in grams is estimated in all blood removed including that withdrawn during sampling. A sample is taken 48 hours after each hemorrhage and hematocrit values and hemoglobin content are measured. Blood volume determinations are made once each week but not less than 48 hours after blood withdrawal of 100 cc. or more. Hemoglobin is estimated in the form of acid hematin colorimetrically compared with a known standard prepared from normal dog blood (5) as measured by the oxygen capacity method of Van Slyke. This standard at 100 per cent is equivalent to a hemoglobin content of 13.8 grams per 100 cc. of blood.

Standard salmon bread

		per cent
Wheat flour	12,000 grams	32.8
Potato flour	6,000 grams	16.4
Bran	2,000 grams	5.4
Sugar	3,000 grams	8.2
Salt mixture	150 grams	0.4
Cod liver oil	1,000 cc.	2.7
Canned tomatoes	2,000 cc.	5.4
Yeast	454 grams	1.2
Canned salmon	2,500 grams	6.8
Water	7,500 cc.	20.5

Directions for baking bread: The following weighed ingredients are put into the bread mixer: One-half the quantity of wheat flour (6,000 grams), the total amount of cod liver oil, dissolved yeast (the yeast is dissolved in one liter of lukewarm water), canned salmon, salt mixture and water. These materials are thoroughly mixed and permitted to stand over night. The next morning the remaining ingredients are added to the liquid mixture and the total mixed for 15 minutes. The dough is then removed from the mixer, slightly kneaded and rolled into 48 loaves, and baked for one hour at a temperature of 300°F. The bread is broken into small fragments and allowed to dry on shallow trays at room temperature before being used in the experiments. The dried bread weights are used in the tables.

Salt mixture minus iron (McCollum and Simmonds (3))

	grams
Sodium chloride	86.5
Magnesium sulphate (anhydrous)	133.0
Calcium phosphate (monobasic)	270.0
Sodium phosphate (monobasic)	173.5
Potassium phosphate (dibasic)	477.0
Calcium lactate	650.0

White bread to make 96 2-lb. loaves
130 lbs. wheat flour
32 qts. water
3 lbs. skim milk powder
3 lbs. yeast (Fleischman's)
2.5 lbs. granulated sugar
2.5 lbs. shortening (lard and fat)
2 lbs. salt

Potatoes (local product) used in the potato basal diet are boiled, peeled and mashed.

Experimental observations. Significant differences in physiological reactions which may be due to apparently insignificant differences in method procedure are well illustrated by table 1. In the earlier years of

TABLE 1

Basal hemoglobin output on salmon bread diet

Average grams hemoglobin per week

DOG NO.	HAND MIXED BREAD					MACHINE MIXED BREAD				
	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935
24–45	3.0	7.7	7.7	5.5	5.5	15.4	13.9		16.4	16.7
23–1		2.4	2.4	3.0	3.0	8.7	8.3	8.3	10.7	13.6
27–238				2.8	5.2	7.4	13.0	7.6	15.8	14.0
27–235				3.6	12.1	17.5	14.5	18.2		
29–65					1.2	15.3	9.8		13.6	12.0
27–234				7.6	7.6	13.5	13.5	6.0		17.8
26–102					2.7	7.8	7.3	6.6	8.6	12.9
27–236					2.2	9.3	5.9	3.3	12.3	12.7
27–240					4.1	13.8	12.4	7.7	12.1	18.0
24–59	8.6	6.0	4.7	1.4	6.6	21.2	9.5			
27–239					2.5	10.0	10.0			
27–241				3.2	2.5	15.7	9.4	10.0		
27–233				11.0	6.0	18.0	11.0			
25–97		7.0	5.3	5.6	4.4	27.0				

the anemia work the salmon bread was mixed and prepared for baking by hand. The hemoglobin production per week on this basal ration was very low—on the average 2 to 6 grams per week. It is certain that the basal output was never fixed as definitely as at present because in the early experiments we never used the 10 week periods to measure accurately the basal output. It is possible that the true level on this hand mixed bread was actually somewhat higher than the figures given (table 1) which were estimated from a considerable number of short bread diet periods.

When the anemia colony was gradually expanded to 30 or more dogs, it became necessary to use a modern motor driven bread mixer and at once it became apparent that the basal hemoglobin output was much larger. To

answer the various questions raised by this change in the basal bread hemoglobin production we used long bread standard periods of 10 to 20 weeks, frequent test feedings with the standard liver ration (300 gm. daily for 2 weeks) and optimum doses of iron (40 mgm. daily for 2 weeks). At length we were forced to the conclusion that the basal bread ration was different while the liver and iron gave the familiar reactions. As the bread ingredients were identical and the only difference in technique was the introduction of the mechanical bread mixer, it seems probable that the bread

TABLE 2

Salmon bread without salt mixture

DIET PERIODS	EXP. PERIOD	FOOD CONS. AVER.	WEIGHT AVER.	BLOOD HEMOGLOBIN LEVEL AVER.	HEMOGLOBIN REMOVED PER WEEK AVER.
Food, grams per day	*wks.*	*per cent*	*kgm.*	*per cent*	*gm.*
Dog 23–1 Bull, male, adult					
Salmon bread 350, salmon 100, Klim 20....	15	100	17.6	50	11.0
Bread minus salt 350, salmon 75, Klim 20..	10	93	18.2	49	10.4
Salmon bread 286, salmon 160, Klim 20....	21	97	17.7	51	13.1
Dog 24–45 Bull, female, adult					
Salmon bread 400, salmon 50, Klim 20.....	12	100	24.6	49	18.2
Bread minus salt 450, salmon 50, Klim 20..	3	100	25.5	51	14.8
Salmon bread 450, salmon 50, Klim 20.....	9	100	23.7	49	16.7
Dog 30–116 Coach, male, adult					
Salmon bread 450, salmon 75, Klim 20.....	11	100	15.3	46	16.6
Bread minus salt 400, salmon 75, Klim 20..	15	100	16.2	46	12.0
Dog 30–117 Coach, male, adult					
Salmon bread 410, salmon 90, Klim 20.....	8	100	14.0	45	12.2
Bread minus salt 425, salmon 75, Klim 20..	15	100	15.5	47	12.0

made by machine is very much more thoroughly mixed and better digested by the dogs. Table 1 shows a prompt jump in the hemoglobin output per week on the standard salmon bread ration when we pass from the hand mixed bread to the machine mixed bread. We note some fluctuations in the basal salmon bread output from year to year. These fluctuations are less conspicuous with the machine mixed bread but are present in all dogs and we have no explanation to offer. In many dogs there is noted a slight but sustained rise in the hemoglobin production from year to year—dogs 23-1, 27-238, 26-102, 27-236—but it is not a conspicuous change and an

occasional dog (29-65) may show the reverse. One might think that in these long continued severe anemias the dog might show evidence of *marrow exhaustion* but we have never observed this—one questionable case was reported (4). When one observes that several dogs show an increased capacity to form hemoglobin on the salmon bread diet as measured year by year, it might be suspected that the hemopoietic mechanism became more efficient in these dogs. At any rate the change is not great and can be

TABLE 3

Salmon bread without bran

DIET PERIODS	EXP. PERIOD	FOOD CONS. AVER.	WEIGHT AVER.	BLOOD HEMOGLOBIN LEVEL AVER.	HEMOGLOBIN REMOVED PER WEEK AVER.
Food, grams per day	*wks.*	*per cent*	*kgm.*	*per cent*	*gm.*
Dog 23–1 Bull, male, adult					
Salmon bread 325, salmon 100, Klim 20	12	100	17.3	42	7.7
Branless bread 325, salmon 75, Klim 20	12	99	17.0	47	5.8
Salmon bread 350, salmon 100, Klim 20	15	100	17.6	50	11.0
Dog 24–45 Bull, female, adult					
Salmon bread 400, salmon 50, Klim 20	8	100	24.1	46	16.5
Branless bread 400, salmon 50, Klim 20	12	100	24.3	46	14.5
Salmon bread 400, salmon 50, Klim 20	12	100	24.6	49	18.2
Dog 29–65 Bull, male, adult					
Salmon bread 275, salmon 100, Klim 20	16	99	13.0	46	14.0
Branless bread 275, salmon 100, Klim 20	4	98	13.3	41	8.0
Salmon bread 300, salmon 115, Klim 20	12	98	13.3	46	15.0
Dog 27–236 Bull, female, adult					
Salmon bread 340, salmon 85, Klim 25	12	92	16.8	49	12.3
Branless bread 375, salmon 85, Klim 20	12	92	17.9	43	8.7
Salmon bread 320, salmon 120, Klim 20	15	96	17.6	44	12.7

controlled adequately by repeating the 10 week standard salmon bread diet periods.

Certain modifications of the standard salmon bread have been adequately tested to ascertain the potency of the various factors going into the bread mixture. Table 2 shows that the presence of the *salt mixture* in the bread mixture has little if any influence upon the hemoglobin production under these conditions. It has been reported previously (8) (see table 2 in that article) that 6 grams of this same salt mixture added daily for 2 weeks to the

basal ration will cause a slight increase in the output of hemoglobin (9 grams on the average per 2 weeks). Whether this slight reaction is due to absorption or to modified internal metabolism is not clear but this reaction is relatively insignificant.

Table 3 shows that *bran* is a factor of some importance in the standard salmon bread. When the bran is left out of the bread mixture the dog does not produce as much hemoglobin as during the control fore and after periods. Some dogs show only a very slight change but most dogs show a definite fall in hemoglobin production which may amount to 30 per cent of

TABLE 4

White bread

DIET PERIODS	EXP. PERIOD	FOOD CONS. AVER.	WEIGHT AVER.	BLOOD HEMOGLOBIN LEVEL AVER.	HEMOGLOBIN REMOVED PER WEEK AVER.
Food, grams per day	*wks.*	*per cent*	*kgm.*	*per cent*	*gm.*
Dog 33–13 Coach, male, adult					
Salmon bread 400, salmon 75, Klim 20.....	18	100	17.4	46	14.4
White bread 420, salmon 75, Klim 20.....	22	97	20.4	47	12.9
Dog 33–85 Bloodhound, female, adult					
White bread 290, salmon 150, Klim 20.....	12	95	17.0	47	7.1
Dog 27–235 Bull, female, adult					
Salmon bread 200, salmon 200, Klim 20....	21	92	14.7	46	16.7
*White bread 175, salmon 175, Klim 20.....	8	89	14.1	49	9.3
Dog 34–220 Mongrel, female, adult					
†White bread 175, salmon 200, Klim 20.....	12	91	14.1	58	8.2

* Salt mixture, 1 gram daily added.

† Cod liver oil 10 cc. plus 1 gram salt mixture daily added.

the base line hemoglobin production. Bran as a supplement to the salmon bread given in large amounts has not yet been tested adequately. Bran contains 13.1 per cent protein which is just about the protein content of the standard salmon bread—12.3 per cent protein.

The *salmon* used in all these experiments is a common grade of canned salmon (trade name "Alaska Pink") and it has been adequately tested as a supplement to the salmon bread so that we are confident that 50 to 200 grams of salmon does not influence appreciably the output of hemoglobin in these experiments. The protein content of this salmon averages 19.5 per cent.

White bread (table 4) has not yet been adequately standardized but it appears from the experiments tabulated that this bread is not as potent for hemoglobin building as the standard salmon bread. The iron content of this white bread is about one-third that of the standard salmon bread which averages 5.5 mgm. per 100 grams. The white bread obviously is not a complete diet and cannot be fed indefinitely as is true for the salmon bread.

TABLE 5

Potato basal ration

DIET PERIODS	EXP. EPRIOD	PROTEIN CONTENT DAILY DIET	FOOD CONS. AVER.	WEIGHT AVER.	BLOOD HEMOGLOBIN LEVEL AVER.	HEMOGLOBIN REMOVED PER WEEK AVER.
Food, grams per day	*wks.*	*gm.*	*per cent*	*kgm.*	*per cent*	*gm.*
Dog 26–164 Coach, male, adult						
Salmon bread 267, salmon 158, Klim 43	15	63.6	99	13.2	46	7.0
Potato basal*	17	25.7	97	12.3	48	14.3
Salmon bread 269, salmon 101, Klim 26	15	52.2	99	12.5	44	6.2
Dog 21–67 Coach, male, adult						
Salmon bread 340, salmon 130, Klim 40	16	67.0	96	16.3	47	10.2
Potato basal†	15	26.6	95	14.6	49	19.0
Salmon bread 347, salmon 107, Klim 40	15	63.4	99	16.4	50	12.8
Dog 30–131 Coach, male, adult						
Salmon bread 435, salmon 100, Klim 40	17	72.9	100	12.9	46	10.9
Potato basal‡	14	25.1	97	12.0	47	12.7
Salmon bread 420, salmon 75, Klim 36	14	66.0	100	13.4	46	9.8

* Potato 532, canned tomato 212, Karo syrup 60, bran 75, cod liver oil 19, cotton seed oil 47.

† Potato 480, canned tomato 203, Karo syrup 60, bran 93, cod liver oil 23, cotton seed oil 47.

‡ Potato 500, canned tomato 230, Karo syrup 75, bran 75, cod liver oil 15, cotton seed oil 50.

The *potato basal ration* is very efficient for the production of hemoglobin in these experiments. It seems certain that the bran is in part responsible but the proteins from both bran and potato must be well utilized. The potato contains 2.5 per cent protein. The potato basal ration contains slightly more than one-third the amount of protein contained in the salmon bread diet (table 5) yet the dogs produce more hemoglobin per week. There is a loss of body weight on the potato basal diet but this change is but slight over a period of 14 to 17 weeks. The iron content of the potato basal ration

as fed is 1.75 mgm. per 100 grams giving a total iron intake per day of 14 to 16 mgm. which is a little less than on a corresponding diet of salmon bread. Obviously the dog utilizes very efficiently both the protein and iron contained in the potato basal ration.

SUMMARY

We have described carefully all method procedures relating to the standard anemic dog and to the study of its capacity to produce new hemoglobin in red cells.

Mechanically mixed bread is better utilized by the anemic dog and the basal hemoglobin output is increased (table 1) as compared with the hand mixed bread of the earlier experiments.

Bran is an important constituent of the salmon bread and is probably responsible for about $\frac{1}{3}$ of the new hemoglobin production in standard experiments.

Potato protein is very well utilized to form new hemoglobin in these experiments.

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FORMATION OF AN EPINEPHRIN-LIKE SUBSTANCE IN AUTOLYZING ADRENAL GLANDS[1]

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The existence of hormones in normal glands, as the same substance extracted from the gland by strong chemical reagents, is still an open question. The menstruum at the disposal of the body cells is only the tissue fluids which differ relatively little from simple isotonic saline solutions. However, in the presence of specific enzymes, the cells, even in a simple menstruum are able to form elaborate products. At present there is no convincing evidence that enzymes are involved in hormone production. Since the hormone of the adrenal medulla (epinephrin) has been extracted and purified to the crystalline state we attempted to liberate the physiologically active substance by simple physical methods comparable to some biological processes and compare the physiological effects and chemical reactions of this substance with the pure crystalline product. The following experiments were accordingly performed.

METHOD. Fresh whole adrenal glands (approximately 20 gram portions) were thoroughly crushed in a mortar with sand (3 grams sand per gram gland) and 50 cc. distilled water, until a very finely divided pulpy mass was formed. This pulp was placed in collodion bags and dialyzed repeatedly against 200 cc. portions of distilled water. The dialysates were removed at intervals varying from 1 to 3 days for 70 days. Dialysis was carried on in an ice chest at about 10°C. When combined dialysate obtained over the short periods amounted to 600 or 800 cc. they were concentrated to 40 or 50 cc. under vacuum and distilled at 33° to 40°C. At the end of 70 days' dialyzing, these fractional concentrates (totaling approximately 1000 cc.) were reconcentrated (as mentioned above) to approximately 100 cc., centrifuged (because of the difficulty in filtering) and the supernatant fluid concentrated (as above) to a final volume of 15 cc. and cooled. Next an equal part of NH_4OH sp.g. 0.9 was cooled and added with constant stirring; allowed to stand for approximately 15 minutes and placed in an ice chest (10°C.) for 24 hours to allow the ppt. to settle. This ppt. was separated from the supernatant fluid by centrifuging and decanting. The decanted liquid was again concentrated to

[1] Preliminary Report. Proc. Soc. for Exp. Biol. and Med. **31:** 534, 1934.

15 cc. (as previously stated) and again ppt. by adding an equal volume of NH_4OH. This procedure was repeated for a third time. After each centrifugation the supernatant fluid was freed from NH_4OH under vacuum at 40° and made up to 100 cc. with distilled water and tested for pressor effect.

RESULTS. Dialyzing the above portions of freshly ground glands for 5 minutes to 70 days, results in a bluish dialysate, 1 to 5 cc. portions of which cause a powerful pressor response when injected intravenously into anesthetized dogs (weighing approximately 10 kgm.) comparable to the effect obtained by injecting ½ to 1 cc. of 1/20,000 P. D. & Co. adrenalin (see fig. 1); also the same powerful pressor response was obtained from the supernatant fluid after three times precipitating with NH_4OH, centrifuging and concentrating.

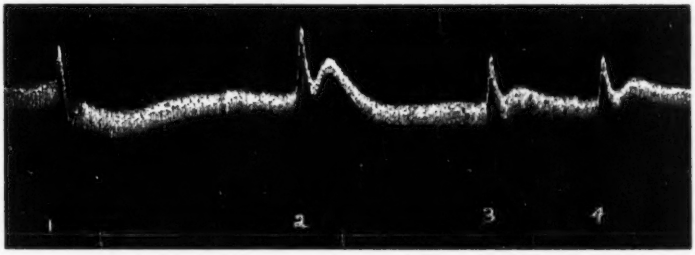

Fig. 1. Comparison of pressor effects obtained by intravenous injections of *1*, 1 cc. adrenalin (Park, Davis & Co.) dilution 1:20,000; and *2*, 1 cc. of dialysate dilution 1:10. (This dialysate dilution was prepared from the combined dialysate obtained by dialyzing 20 grams adrenal glands for 70 days and concentrating to 750 cc.; then diluting 1:10 and injecting 1 cc. of the dilution.) At *3*, 1 cc. of P. D. & Co. adrenalin 1:20,000 was again injected. At *4*, 1 cc. of dialysate dilution 1:20 was injected. Note comparable effects of *1*, *3* and *4*.

After dialyzing for several days the glandular contents of the dialyzing bag were well washed in distilled water until the washings gave no pressor response. Then the gland mass again placed in the dialyzing bag and dialyzing again allowed to proceed for 1 to 3 days and the dialysates again tested for pressor principle. A powerful pressor principle could be detected up to the 70th day, clearly indicating that as autolysis proceeded pressor principle formed until almost complete disintegration of the glandular substance occurred.

The precipitates obtained after adding the NH_4OH were suspended in 80 cc. distilled water, washed well, centrifuged and decanted (this repeated 3 times) then ground in a mortar with 100 cc. distilled water. One cubic centimeter portions of this dispersion and fine suspension were injected intravenously into anesthetized 10 kgm. dogs. Neither pressor nor de-

pressor effects were obtained, indicating that the powerful pressor principle which we obtained from autolyzing adrenal glands is not precipitated by the same chemical reagent (NH_4OH), and under the same conditions as epinephrin.

If autolysis of the crushed adrenal glands during the dialysis be held in abeyance by adding potassium cyanide to the ground glands in the dialyzing bags, no pressor principle could be detected in the dialysate. On the other hand K C N added to the dialysates obtained from untreated glands does not affect the pressor activity, neither does it affect the pressor activity of P. D. & Co. adrenalin. Boiling for 1 minute does not destroy the pressor effect of the dialysate, neither does digestion with pepsin or trypsin destroy it. Passing the dialysate through animal charcoal removes the pressor principle, as does adsorption on aluminum hydroxide.

CONCLUSION

Autolysis of crushed, whole adrenal glands gives rise to a powerful pressor substance which may be obtained by dialysis. This substance gives all the ordinary chemical tests for adrenalin, but differs from it in that it is not precipitated by concentrated ammonium hydroxide. It does not give a biuret reaction; is not destroyed by boiling for 1 min.; is adsorbed by animal charcoal and aluminum hydroxide and does not lose its physiological effect if exposed to air at room temperature for 30 days. Glacial acetic acid does not destroy its pressor principle. Inhibiting autolysis of the crushed adrenal glands by the addition of potassium cyanide prevents the formation of the pressor substance as determined by chemical and physiological tests.

The pressor substance contained in the dialysate is not precipitated by concentrated NH_4OH, although a heavy flocculent precipitate forms. The mother liquor from this precipitate contains a powerful pressor substance.

The formed pressor substance may be removed from the crushed adrenal glands by repeated washing. Further autolysis, however, results in the formation of more pressor substance, which continues to form until practically complete disintegration of the gland substance has occurred.

THE ACTION OF A SYNTHETIC OESTROGENIC AGENT ON THE ANTERIOR PITUITARY OF THE CASTRATED FEMALE RAT[1,2]

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It has recently been demonstrated by Cook, Dodds and Hewett (1), Cook, Dodds, Hewett and Lawson (2) and Dodds (3) that certain synthetic condensed-ring compounds somewhat similar in structure to oestrin possess the capacity to induce oestrus when injected into the castrated female rat. Both the natural oestrins and the synthetic oestrogenic compounds are derivatives of phenanthrene. One particular synthetic compound, 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene, was found to be an especially effective oestrogenic agent; injection of 0.05 mgm. of this substance induced oestrus in 50 per cent of the test rats injected. This substance is one of a series of dibenzanthracene diols, derived from the carcogenic hydrocarbon, 1:2:5:6-dibenzanthracene. Since this substance, 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene, is an effective oestrogenic agent, we have carried out a series of experiments to ascertain if this synthetic compound possesses another important physiological property of the natural oestrin: viz., the capacity to prevent the structural changes which occur in the anterior hypophysis of the female rat following castration (4).

Twenty mature female rats were castrated and sacrificed 30 days later. Throughout the castration period these rats received daily subcutaneous injections of from 0.2 to 0.4 mgm. of 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene (dissolved in sesame oil). For control material we have utilized studies made previously on the anterior pituitaries of 43 female rats castrated for 30 days (5) (6). Vaginal smears

[1] These studies were made possible by the cooperation of Dr. E. C. Dodds of the Courtauld Institute of Biochemistry of the Middlesex Hospital Medical School, London. Dr. Dodds furnished us with the synthetic oestrogenic agent, 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene, used in this work. Dr. Dodds was also kind enough to read the manuscript previous to publication.

[2] These studies were aided by grants from the Grants-in-Aid Committee of the National Research Council and from the Division of Medical Sciences of the Rockefeller Foundation.

made daily on the injected rats revealed that these rats stayed in a continuous or almost continuous oestrus. At autopsy it was found that injections of the synthetic oestrogenic agent induced a rather marked weight increase in the pituitaries of the experimental rats as compared with those of the 30-day castrated control rats (table 1). The mean pituitary weight of the injected rats was 14.2 mgm., and that of the 30-day castrated controls was 9.7 mgm.

It is well established that castration in the rat results in definite structural changes in the anterior hypophysis. In recent quantitative studies, Ellison and Wolfe (5) reported that castration in the mature female rat resulted in an early increase in the size and the relative numbers of the basophiles which reached an apex approximately 30 days after castration. Differential cell counts revealed that the mean level of the basophiles at this period after castration was 13.4 per cent. At this time a few typical signet-ring castration cells were found in 33 of 43 pituitaries studied. There was also a much less evident increase in the percentages of the eosinophiles in the 30-day castrates. In table 1, detailed quantitative data are given on the pituitaries of 30-day castrates; for comparison, similar data are given on the anterior pituitaries of 143 normal female rats killed during the oestrual cycle. These two groups served as 30-day castrate controls and normal controls, respectively.

Serial sections of the pituitaries of rats receiving the synthetic oestrogenic agent were cut and differential cell counts were made on representative sections. Structural changes which normally occur in the anterior hypophysis following castration were completely prevented. There was no evidence of any increase in the numbers of the basophiles; in fact, practically all these cells were completely degranulated and the relative levels were reduced to the extremely low mean of 2.1 per cent. The mean level of the basophiles in the 30-day castrate controls was 13.4 per cent, while in normal female rats killed during the oestrual cycle the mean was 4.1 per cent (table 1). The non-granular basophiles in the injected rats appeared as large cells with a slight blue cytoplasm which in some instances was fragmentary. The negative image of the Golgi apparatus was often visible.

There was also a marked degranulation of the eosinophiles in the injected rats and the relative level of these cells was reduced far below that found in the untreated 30-day castrates, and considerably below that found in normal cycle females (table 1). Partially degranulated eosinophiles were usually somewhat swollen; the granules remaining in the cell took a light stain with orange G. The negative image of the Golgi apparatus was often hypertrophied. Correlated with the decreases in the percentages of the eosinophiles and the basophiles in the rats receiving the synthetic oestrogenic agent, there was a marked increase in the levels of the chromophobes. As we have reported previously, morphologic evi-

TABLE 1

The quantitative data are arranged in statistical form

	FREQUENCY INTERVALS	FREQUENCY DISTRIBUTION			MEAN LEVELS IN PERCENTAGE		
			30-day castrates			30-day castrates	
		Normal controls	Controls	Synthetic oestrogenic agent	Normal controls	Controls	Synthetic oestrogenic agent
	per cent						
Eosinophiles	15.0–19.9			2	34.2	41.3	24.2
	20.0–24.9	4		10			
	25.0–29.9	14		8			
	30.0–34.9	66	1				
	35.0–39.9	49	16				
	40.0–44.9	9	17				
	45.0–49.9	1	9				
Basophiles	0.0– 1.9			9	4.1	13.4	2.1
	2.0– 3.9	45		11			
	4.0– 5.9	74					
	6.0– 7.9	14					
	8.0– 9.9		2				
	10.0–11.9		7				
	12.0–13.9		18				
	14.0–15.9		12				
	16.0–17.9		4				
Castration cells	None	143	10	20			
	0.0– 1.9		28				
	2.0– 3.9		3			1.1	
	4.0– 5.9		1				
	6.0– 7.9		1				
	8.0– 9.9						
Chromophobes	30.0–34.9				61.7	44.2	73.7
	35.0–39.9		10				
	40.0–44.9		11				
	45.0–49.9	1	16				
	50.0–54.9	4	6				
	55.0–59.9	41					
	60.0–64.9	69					
	65.0–69.9	28		2			
	70.0–74.9			12			
	75.0–79.9			6			
	80.0–84.9						
Mean pituitary weight, mgm.		10.2	9.7	14.2			

dence as well as quantitative cell counts indicate that, after losing their granules, the eosinophiles and the basophiles revert to chromophobes. The above morphologic changes induced in the anterior pituitaries of the

injected castrated female rats were in every way similar to those induced by the natural oestrins. Quantitatively, they were much more marked than the reaction obtained by the injection of 25 units of oestrone[3] daily for the same period; they were considerably less marked than the changes induced by the daily injection of 200 units of the benzoic ester of dihydroestrin[4] for the same period (4).

Representative sections of the uteri and vaginae of these rats were cut; the vaginae of all were either stratified or stratified and cornified. The lining epithelium of the uteri was high pseudostratified; in two instances there was found a cystic dilatation of the uterine glands; a condition induced previously by injection of the natural oestrins (7) (8) (9).

Discussion. The earlier studies of Cook and his associates demonstrated that 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanththracene possesses the capacity of inducing changes in the vaginae and uteri of castrated rats characteristic of oestrus; an important physiological property of the natural oestrogenic substances. These studies indicate that this synthetic oestrogenic agent induces other physiological reactions which are commonly induced by the natural oestrins. Injection of this material increases the weight of the pituitary in castrated female rats; induces degranulation of the eosinophiles and basophiles and completely prevents the structural changes which normally occur in the anterior lobe following castration. It also induces cystic dilatation of the uterine glands in some instances. It is a matter of considerable significance that a synthetic oestrogenic compound, differing considerably in structure from the natural oestrins, is able to reproduce these physiological reactions in the organism usually induced by the natural oestrogenic substances.

SUMMARY AND CONCLUSIONS

Twenty mature female rats, castrated for 30 days, received daily injections of 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene throughout the castration period. Such animals remained in either a continuous or almost continuous state of oestrus. The pituitary weights were increased as compared with those of untreated 30-day castrate controls. The structural changes which normally occur in the anterior hypophysis of the female rat after castration were prevented. Extreme degranulation of the basophiles and moderate degranulation of the eosinophiles were induced. Cell counts revealed that the eosinophiles and the basophiles were reduced in relative percentages, the chromophobes

[3] Amniotin was used. This material was furnished by E. R. Squibb and Sons through the courtesy of Dr. J. J. Durrett.

[4] Progynon-B, the benzoic ester of dihydroestrin, was used. A portion of this material was furnished gratuituously by the Schering Corporation, through the courtesy of Dr. Erwin Schwenk.

increased. In two instances, cystic glandular hyperplasia of the uterus was produced.

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INSENSIBLE WATER LOSS IN RELATION TO WATER INGESTION IN MAN

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The present study attempts to ascertain under carefully controlled conditions whether factors other than energy metabolism might modify the rate of evaporation from the skin and lungs; and to define as far as possible the rôle of the insensible perspiration in water-regulation. The work of Soderstrom and DuBois (1917), Benedict and Root (1926), Levine and Wilson (1927), and others, indicated a heat-regulating rôle as the chief function of the insensible perspiration. Is it possible that under other conditions another function may be served by the same process?

It has been frequently reported that the insensible loss by evaporation is increased in man by water drinking (Erismann, 1875; Moog and Nauck, 1921; Bratusch-Marrain, 1929; Manchester, Husted and McQuarrie, 1931; Czike, 1934). Others have not found any change (Peiper, 1889; Laschtschenko, 1898; Daniel and Högler, 1922; Heller and Natanson, 1929). All these investigators used indirect methods of measurement, or long periods of time. Work comparable in method with ours was reported by Levine, Wilson, and Kelly (1929), Gereb and Laszlo (1931), Chrometzka and Schweder (1931), and Hovland (1935b). All but the last named found no consistent increase in evaporation rate from the body as a whole.

Some workers such as Schlüter (1925) indicated that *reduction* of the fluid intake caused a lowering of the amount of water eliminated through the insensible perspiration. Levine and Wyatt (1932) found that in sixteen clinically dehydrated infants the basal insensible loss of weight averaged 11 per cent below that of the same group after recovery. More recently Newburgh and Johnston (1934) concluded that dehydration amounting to as much as 6 per cent of the body water failed to produce any significant change in the rate of insensible loss as measured indirectly.

These conflicting results are sufficient evidence to indicate the need of carefully controlled studies of the effect of various degrees of hydration and of dehydration upon the insensible perspiration of the normal adult and infant. It should be possible to define the conditions under which each result is to be found.

METHODS. The experiments extended over a period of six months, and a total of 50 experiments were performed on normal adults, chiefly upon three for whom adequate control data were available. All experiments were routinely carried out with the subject in a "basal" condition. This insured that the results obtained would not be complicated by effects of exercise, food, nor a markedly high or low respiratory quotient, for as shown by Johnston and Newburgh (1930) water vapor constitutes 93 per cent of the total insensible loss when the R.Q. is 0.82, this respiratory quotient being characteristic of individuals in a basal condition. The temperature under which the experiments were performed was always maintained between 25° and 28°C., the relative humidity between 50 and 60 per cent. The usual temperature was 26°, the usual humidity 50 per cent. These conditions of temperature and humidity were found to be entirely satisfactory in that sweating did not occur, and in any one experiment the conditions were much more constant than the limits indicated above.

The rate of insensible loss was measured with a Sauter balance having a capacity of 100 kgm. and a sensitivity of one-tenth gram. The subject, wearing only a light pair of cotton trunks, lay directly upon the all-metal bed suspended from one arm of the balance, and was nearly balanced by weights applied to the weight pan. At the moment when an exact balance occurred, a stop watch was started. An additional 5 or 10 gram weight then was added to the subject's side of the balance and this side was thus overbalanced by a definite weight. By observing the time as recorded by the stop watch for the subject to lose this weight, a measure of the rate of the insensible perspiration was obtained every 7 to 25 minutes.

The limit of error of the method is set by the time of one complete swing of the pointer of the balance. This was approximately 25 seconds. With the average normal subject having an insensible loss of 30 grams per hour, such an error amounted to 4 per cent when the 5 gram weight was used and only about 2 per cent when the 10 gram weight was used.

In plotting the graphs, each individual point represents the rate of insensible perspiration as determined by a loss of weight which terminated at the time indicated on the abscissae. The points for the skin temperatures, rate of urine excretion, pulse rate, and for body weight were plotted in the same manner. The rate of urine excretion when determined was ascertained by having the subject rise and empty the urinary bladder at intervals. The subject also rose to drink the various solutions in all but certain special tests. Except for these changes the recumbent position was maintained throughout an experiment. Urine volumes were measured, and specific gravities were determined with a spindle. Temperature measurements were continuously recorded in a number of experiments by means of resistance thermometers (Burton, 1934) placed on the chest, arm, and leg of the subject.

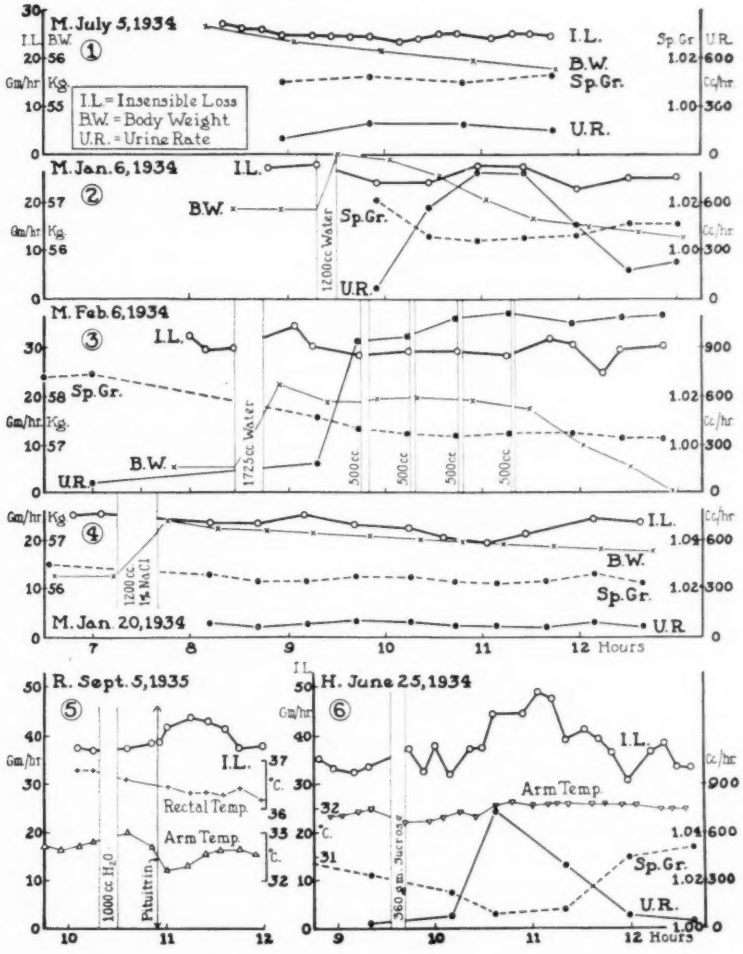
EXPERIMENTAL RESULTS. Several graphs are presented which are representative of the results obtained. A normal or control period, sufficiently extended to assure that a basal rate of insensible perspiration had been attained, always preceded the experimental period. The experimental period began with the ingestion and continued until the basal rate had been reattained. Thus the average of the experimental period could be compared directly with that of its own control period.

Controls. Seven normal or control experiments were performed on three adult subjects to determine the experimental errors of the method employed and the uncontrollable variations to be expected. Figure 1 represents such an experiment in which the rates of insensible perspiration were remarkably constant throughout.

Water ingestion. In fourteen experiments large volumes of water (at 34°–37°C.) were ingested. In this series an attempt was made to ascertain if single ingestions of water, by temporarily increasing the water content of the body, would also increase the rate of insensible perspiration. A diuresis began within a period of thirty minutes to one hour after such ingestions, and figure 2 shows a tendency, occasionally found, to a slight increase in rate of insensible loss which is not considered significant. In most experiments of this type no significant change in the rate of insensible perspiration was detected; but since in several a small temporary increase of rate followed the ingestion, a group of experiments was carried out to find whether this occurrence could be controlled. It was found that if the water was drunk through a tube while the subject was prone, the rise of rate could be avoided. This important point was apparently not tested by Hovland (1935b) and others who reported a positive effect of water ingestion.

In three experiments by continuous ingestions of large volumes of water, an abnormally high rate of urine excretion was maintained over a period of two or three hours. Figure 3 is typical of the rates of the insensible perspiration obtained with continuous water ingestions. A total of 3700 cc. of water was ingested in this experiment, yet no change in rate of insensible loss was observed, the average for the experimental period being within 3 per cent of that for the control period.

Saline ingestion. A group of four experiments similar in routine to the water drinking experiments were carried out, substituting 1 per cent sodium chloride solutions. It is well known that by the use of this salt in solution of proper concentration diuresis is prevented and the ingested liquid stored in the body for an appreciable time. Figure 4 is representative of results obtained; ingestions of a 1 per cent sodium chloride solution prevented diuresis, yet despite the retention of water and consequent increased hydration of the body, the rate of insensible perspiration was not altered.



Figs. 1–6. The first four figures are plotted so that the clock hours were synchronous for all. The fluid ingested was in every case at 34° to 37°C. Specific gravity (Sp. Gr.) is that of urine. The subject of the experiment is indicated by a letter preceding the date. 1. Control experiment. 2. Single ingestion of water. 3. Five successive ingestions of water. 4. Ingestion of dilute sodium chloride solution. 5. Ingestion of water followed by intramuscular injection of 12 units of posterior pituitary extract. 6. Ingestion of sucrose with 750 cc. of water.

Water and pituitrin. In eight experiments, following the ingestion of a large volume of water, an intramuscular injection of 0.5 to 0.75 cc. of surgical pituitrin was given. This procedure inhibited the diuresis which the

water would otherwise have produced, and insured at least a temporary retention of the water and an increased state of body hydration. Six of these experiments showed an increase in the rate of insensible perspiration immediately following the pituitrin injection; figure 5 being representative. In some of the instances this increase might be due to reflex effects of some kind. In two experiments such an increase in the rate was not present; in one of them the water was ingested a whole hour before the pituitrin was injected, which might be a significant variation in procedure. The increase in rate of insensible loss lasted only an hour at most, while the increment of the body's water content prevailed for several hours. It is uncertain whether the increase of insensible loss is attributable to the hydration of the body or not.

In order to ascertain if the changes produced by pituitrin would be of a magnitude sufficient to alter the insensible perspiration, in two experiments pituitrin alone was injected. These experiments demonstrated that pituitrin did not increase the rate of insensible perspiration.

Sugar ingestion. With the object of contrasting with the foregoing the effects of substances stimulating the energy metabolism, seven experiments were performed in which large quantities of sucrose in water (at 37°C.) were ingested. Qualitative tests for reducing sugars were made on the urine excreted during these experiments, in an effort to correlate the appearance of urinary sugars with observed changes in skin temperature and insensible perspiration. In the course of the work it became evident that individual differences in rates of absorption and metabolism and in renal thresholds made such a correlation impossible. The results of these sucrose ingestion experiments are represented by figure 6. The marked rise in trunk temperature, the slight rise in leg and arm temperatures, the increased pulse rate—all seem to be correlated with an increased rate of insensible perspiration. The average for the control period was 31.0 grams per hour and that of the experimental period 38.0 grams per hour. The percentage change in rate of insensible loss corresponds approximately to the percentage increase of heat production that has been measured by numerous investigators after ingesting large quantities of sucrose. In every experiment of this type the results were similar, though there were some differences in the time required for the stimulating effect to become most pronounced, due, perhaps, to different rates of absorption, to different stimulations of metabolic increase, and to different degrees of circulatory response (Burton and Murlin, 1935). Similar experiments were reported by Hovland (1935a, 1935b) after ours had been completed.

Dehydration. Three dehydration experiments were performed to determine if depletion of body water alters the rate of insensible perspiration. The routine followed in such experiments consisted in permitting the subject to eat dry, solid foods so as to insure a caloric intake sufficient

to meet his energy requirements, but all liquids were restricted. By this method a progressive dehydration was produced. Vigorous exercise to produce sweating and thus increase the rate of loss of body water was also resorted to. In all three experiments typical subjective signs of a moderate dehydration were present. The results of one experiment are shown in table 1. In this experiment a loss of 4.7 kgm. represents, if one assumes that 70 per cent of the body weight is water, a loss of approximately 7.9 per cent of the total body water of the subject. With such a loss, a marked decrease in the rate of the insensible perspiration occurred. This decrease occurred in both experiments where the loss of body water exceeded 6 per cent, but in the other experiment where the percentage of body water lost did not exceed 6 per cent of the total body water, such a significant decrease in the rate of insensible perspiration was not observed.

TABLE 1

Dehydration experiment, subject M. C. N.

DATE	BODY WEIGHT	INSENSIBLE LOSS	MEAN DEVIATION
	kgm.	*grams per hour*	
July 16	85.239	43.9 (av. of 5 periods)	(±) 1.5
July 17	81.557	39.2 (av. of 9 periods)	(±) 2.3
July 18	80.517	33.6 (av. of 9 periods)	(±) 1.2

Discussion. In the series of experiments in which the hydration of the body was increased temporarily by drinking water or salt solutions, no significant or consistent change in the rate of insensible perspiration could be detected with the method employed. The experiments in which an abnormally high rate of urine excretion was maintained and where presumably the kidney was functioning at its highest level, showed no compensatory tendency of the skin to change its rate of water loss in order to facilitate the removal of the excess water from the body. There appears to be no doubt that both the blood and other tissues are diluted for a period of one to two hours by a single ingestion of water (Baldes and Smirk, 1934). It is barely possible to argue, however, that the increase of bodily hydration so produced is too small to be an effective influence upon the rate of insensible perspiration.

When salt solutions (1 per cent NaCl) were ingested, the diuresis being avoided and the liquid thus retained for some time in the body, no change in the rate of insensible perspiration was observed. The averages for the control periods were almost identical with the averages of the experimental periods. Chrometzka and Schweder (1931) also tested the effect of pituitrin and of 300 cc. of 0.85 per cent sodium chloride upon the insensible perspiration, using approximately the same procedure as ours in measuring the insensible loss; both administrations had no effect.

The sucrose experiments evidently involved factors of heat regulation, for the ingestion of 300 to 500 grams of sucrose is well known to increase by 20 to 50 per cent the rate of heat production and to increase the skin temperature. Both of these increases are correlated closely in point of time. These results confirm the observations of DeRudder (1928) made on infants; those of Chrometzka and Schweder (1931) who found that glucose increased the evaporation rate in adult subjects; and those of Hovland (1935a) who measured increases of evaporation rate after meals. The skin temperature records are valuable in experiments of this type for they furnish additional clues to the physiological changes that are responsible for the observed effects (Burton and Murlin, 1935).

The question of how dehydration affects the rate of insensible perspiration is far from being answered by the experiments reported. It was observed in agreement with the report of Newburgh and Johnston (1934) that a loss of body water not exceeding 6 per cent did not markedly alter the rate of the insensible perspiration. Lowered rates of insensible perspiration prevailed when the loss of body water exceeded 6 per cent. The rate of insensible perspiration is dependent upon the state of body hydration only perhaps in extreme degrees of dehydration and in certain abnormal conditions resulting from disease. It may be suggested that the mechanism involved in the appearance of a dehydration fever is an impairment of the circulation to the skin rather than a direct impairment of evaporation. This particular aspect of the insensible perspiration problem has clinical importance and deserves further investigation.

The calculation of the insensible water loss by means of empirical formulae has been criticized by Peters, Kydd and Lavietes (1933) and Lavietes (1935); and the inaccuracies in the indirect methods of estimating insensible losses are shown by them to be too great to allow the use of these methods in detecting changes due to modifications of water balance. The necessity for a rigid control of temperature and humidity in evaporation studies should be emphasized; the failure to regulate these within narrow limits accounts for some of the conflicting results obtained by various workers. Another factor that may account for the disparity of results upon insensible perspiration is the rapidity with which basal changes in the rate of insensible perspiration might occur. Unless the rate was determined at short intervals, the fluctuations due to any experimental procedure would not be significant. Further cases reported in the literature of increased evaporation occurring after water drinking may possibly be explained as due to 1, the effort of change of posture; 2, the ingestion of water that was too cold or too warm; or 3, the failure to carry out the experimental procedures upon subjects in a "basal" condition. In the case of dehydration experiments the tendency of the sub-

ject to lower his activity enters, as Newburgh and Johnston (1934) pointed out.

The experimental data obtained in this study indicate clearly that, for the normal adult in the basal condition, the rate of insensible perspiration is not so much a function of the state of hydration as it is of the rate of heat production. However, in extreme degrees of dehydration, changes in the rate of insensible perspiration may occur without corresponding changes in the energy metabolism. Possibly the administration of water and pituitrin together produce the extreme degree of hydration required to influence the rate, for in this circumstance alone, of the several means of hydration tested, was insensible loss increased temporarily.

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SUMMARY

1. In normal adult subjects under basal conditions, ingestions of large volumes of water (at 37°C.) produced diuresis but failed to alter the rate of insensible perspiration. Even in periods of maintained diuresis, produced by continuous ingestions of water, no change in the rate of insensible perspiration occurred.

2. Large volumes of 1 per cent sodium chloride solutions were retained for considerable time after ingestion but also failed to alter the rate of insensible perspiration.

3. Pituitrin injections alone did not increase the rate of insensible perspiration. Ingestions of large volumes of water followed by intramuscular injections of pituitrin produced inconstant results, 6 out of 8 experiments, however, exhibiting temporarily increased rates of insensible perspiration.

4. Sucrose ingestions invariably produced marked increases in the rate of insensible perspiration with increased skin temperatures and increased pulse rates.

5. Dehydrations exceeding 6 per cent of the total body water lowered the rate of insensible perspiration. Dehydrations of lesser magnitude did not significantly change the rate.

6. It is concluded that insensible perspiration is not primarily a means of water regulation.

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THE EFFECT OF O_2-LACK, VARIATIONS IN THE CO_2-CONTENT OF THE INSPIRED AIR, AND HYPERPNEA ON VISUAL INTENSITY DISCRIMINATION

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In a previous paper Gellhorn and Spiesman showed that under the influence of O_2-lack, CO_2-excess and hyperpnea the latent period of after-images was considerably lengthened. This seemed to be an indication of a decrease in visual excitability comparable to that found in similar experiments on hearing (Gellhorn and Spiesman, 1935). Since the after-image is a rather special phenomenon it was thought desirable to extend the study to other visual functions. Intensity discrimination was chosen as an important physiological criterion of visual function. For this reason a new series of experiments was carried out in which the sensitivity of the visual apparatus was measured directly by the determination of the sensitivity of the human eye to differences in intensity.

METHOD. Two Masson discs (cf. Helmholtz, 1911) were rotated 1 meter in front of the eyes of the experimental subject, whose head was held in position with a head holder. The various steps in brightness were as follows:

1. 1/42 2. 1/63 3. 1/84 4. 1/105 5. 1/126 6. 1/147
7. 1/167 8. 1/188 9. 1/209 10. 1/230.

Numbers 1, 3, 5, and 7 were seen on the left, and numbers 2, 4, 6, and 8 were seen on the right disc. The experimental subject indicated through the fingers of his hand how many of the grey rings he could see. In control, as well as in the experiments with various gas mixtures, the experimental subject inhaled air or the gas mixture through a mouthpiece from a Douglas bag. After a number of controls were obtained the rubber tubing was connected to the Douglas bag containing a suitable gas mixture but this procedure was concealed from the subject. At the end of the experiment when the breathing of the gas mixture was discontinued and air was readmitted, the subject was not aware of any change. The observations were made with both eyes. In experiments carried out with artificial pupils in front of both eyes the same results were obtained as when no precautions were taken to keep the pupillary diameter constant.

RESULTS. Forty experiments were carried out with uniform results. The first group concerns experiments on O_2-lack where 8 to 10 per cent O_2 in N_2 was inhaled for approximately 10 minutes. The determination of the threshold for intensity discrimination was carried on in 1 or 2 minute intervals. A typical result is seen in figure 1 in which three experiments with varying concentrations of O_2 were carried out on the same subject. It is evident from the figure that the threshold for intensity discrimination increases with decreasing O_2 tension. The effect with 10 per cent O_2 is very small and only temporary. It may be completely absent in other cases.

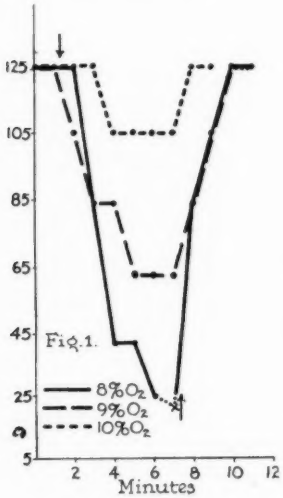

Fig. 1

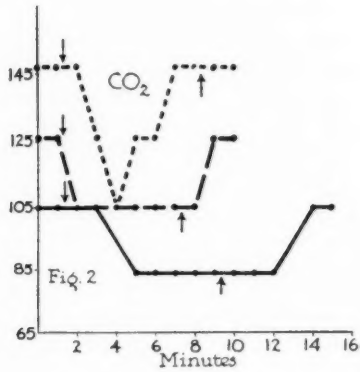

Fig. 2

Fig. 1. Influence of various degrees of O_2-lack (between ↓ and ↑) on the intensity discrimination in the same subject. Ordinate: Reciprocals of the threshold for intensity discrimination. Abscissa: Time in minutes.

Fig. 2. Influence of 6 per cent CO_2 (between ↓ and ↑) on intensity discrimination.

The effects of 8 and 9 per cent are considerable but comparatively quickly reversible upon readmission of air. In the experiment with 8 per cent the last determination during the O_2-lack period could not be carried out because the subject fainted in the experiment illustrated, but one minute later he was able to make the observation and showed, after the readmission of air, a considerable improvement in intensity discrimination.[1] A few examples of the effects of O_2-lack on the visual intensity discrimination (table 1) show that this effect occurs regularly with different individuals,

[1] If the subject could see none of the grey rings so that his intensity discrimination was less than 1/42 it was arbitrarily marked as 1/25 in the graphs.

but that there are individual differences in the greatness of loss during O_2-lack, as well as in regard to the speed of recovery.

A second group of experiments dealt with the effects of CO_2 on the visual intensity discrimination. In these experiments air containing 6 per cent CO_2 was inhaled. Typical experiments (fig. 2) indicate that under these conditions the visual excitability for intensity discrimination decreases but the effects are by far smaller than under O_2-lack. As to the speed of recovery, significant individual differences are observed which are illustrated in figure 2. In the top figure the original intensity discrimination is regained during the time the subject inhales the CO_2-air mixture. In

TABLE 1

The influence of O_2-lack on intensity discrimination

SUBJECT	CONTROL	O_2-CONCENTRATION	O_2-LACK	CONTROL AFTER O_2-LACK
		per cent		
Sp.	*1. 105†	9	2. 105 7. 84	1. 84 5. 105
	2. 105		5. 105 8. 84	3. 84 6. 105
B.	1. 105	$8\frac{1}{2}$	1. 84 3. 63 5. 63	1. 63 3. 84 5. 105
	2. 105		2. 63 4. 63	2. 84 4. 105
Bl.	1. 126	9	1. 105 4. 105 6. 84 8. 105	1. 126
	2. 126		2. 105 5. 84 7. 84	2. 126
L.	1. 126	9	1. 126 4. 84 6. 105 9. 105	1. 105 3. 126
	2. 126		3. 105 5. 105 8. 84	2. 126
H.	1. 126	9	2. 84 5. 84 8. 63	1. 84 3. 105 6. 126
	2. 126		3. 84 7. 63 9. 63	2. 105 4. 105 7. 126

* Time in minutes.

† The figures represent the reciprocals of the thresholds for intensity discrimination.

the experiments reproduced in the middle figure it takes 2 minutes, and in that of the lower figure 4 minutes for a complete recovery.

The last group deals with the effect of hyperpnea on intensity discrimination. The experiments were carried out as in the preceding studies by allowing the subjects to breathe maximally at the rate of 35 per minute for 2 minutes. The test was made at the end of the first and of the second minute of hyperpnea, and thereafter the recovery was followed at intervals of 1 minute. Typical experiments reproduced in figure 3 show that hyperpnea is accompanied by a reversible loss in sensitivity of intensity discrimination.

Here, as in our former experiments on hearing and negative after-images,

it was found that after O_2-lack and hyperpnea not infrequently a supernormal phase was observed. It is interesting to note that this phenomenon is particularly marked in some individuals in whom it occurs regularly after a decrease in intensity discrimination had been established through O_2-lack and hyperpnea (fig. 4).

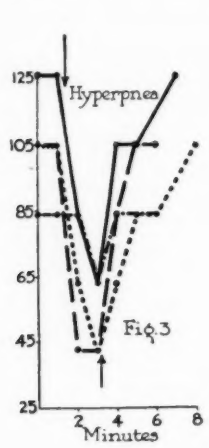

Fig. 3

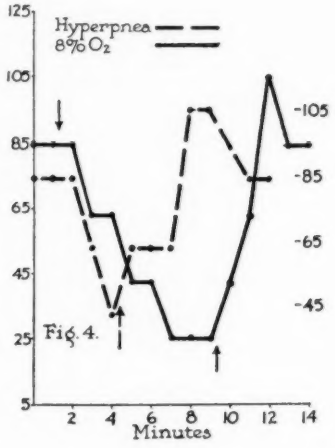

Fig. 4

Fig. 3. Influence of hyperpnea (between ↓ and ↑) on intensity discrimination.

Fig. 4. The occurrence of a supernormal phase in intensity discrimination after O_2-lack and hyperpnea. The left ordinate refers to the O_2-experiment, the right ordinate to the hyperpnea experiment.

Discussion. The results of our experiments are strictly comparable to those obtained in our previous studies on hearing and negative afterimages. They show that O_2-lack, CO_2-excess and hyperpnea decrease the excitability of the visual apparatus reversibly. Depending on the severity of the effect the recovery may be immediate or may last a number of minutes. A supernormal phase may sometimes be observed and is not specific for O_2-lack, as we have previously noted in our hearing experiments. The observations confirm some experiments of Schubert (1933), who found a decrease in intensity discrimination under O_2-lack. In addition they show that these effects are not typical for O_2-lack but are also obtained as the result of hyperpnea and when CO_2-rich gas mixtures are inhaled.

Concerning the relationship of these findings to the circulatory changes studied by Cobb (1933), Fremont-Smith (1931), Lennox (1932), Gibbs (1935) and Schmidt (1934, 1936) reference may be made to our previous papers. The question, however, as to what part of the retino-cerebral system is primarily responsible for the alteration in intensity discrimination

may be discussed briefly. Hecht (1936) and Wright (1935) who recently reinvestigated the problem of the constancy of the quotient $\frac{\Delta I}{I}$ over a wide range of intensities and in different states of adaptation came to opposite conclusions regarding the possible mechanism involved. Hecht assumes it to be retinal, whereas, according to Wright, it "must be due to a post-adaptation process in the chain of events connecting stimulus and sensation." Although we believe that Hecht has conclusively shown that Wright's interpretation of his own data is incorrect and supports rather than contradicts Hecht's photochemical (and therefore retinal) theory of intensity discrimination, it does not necessarily follow that factors like O_2-lack, etc., influencing intensity discrimination have an exclusively peripheral point of attack (cf. also Hecht, p. 16 annotation). It seems to be certain that the intensity discrimination as well as any other visual function can be altered by modifying peripheral (retinal) as well as central processes.

There is, however, a significant difference between the visual apparatus on one side and the auditory and vestibular apparatus on the other side. The fact that the action currents of the vestibular nerve have ceased (Mowrer, 1935) while nystagmic movements may continue for a considerable period of time shows conclusively that the nystagmus is due to after-discharges of those brain stem elements which have been stimulated by the vestibular apparatus. Any alterations in nystagmus must, if the stimulation of the vestibular apparatus is kept constant, be of central and not of peripheral origin. The lack of sensitivity of the cochlear apparatus to O_2-lack which is proven by the persistence of the electrical cochlear response for some time even after the death of the animal (Davis, 1935) rules out this end organ as the point of attack in our O_2-lack experiments. We may therefore state that the influence of O_2-lack, etc., on hearing and nystagmus is exclusively of cortical and subcortical origin respectively. In the case of vision, on the other hand, the presence of ganglion cells in the retina as well as in the cortex makes it probable that both structures are sensitive to O_2-lack, but does not allow one to carry the analysis further.

CONCLUSIONS

O_2-lack (8–10 per cent O_2–N_2 mixtures), 6 per cent CO_2-air mixtures and hyperpnea produce a reversible decrease in visual intensity discrimination. The magnitude of the change and the duration of the recovery depends upon the degree of O_2-lack. Under all three conditions a supernormal phase is sometimes observed after the admission of air. The experiments prove that the excitability of the auditory and of the visual apparatus are influenced in a similar manner by hyperpnea, O_2-lack and CO_2-excess.

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PERIODIC MICTURITION IN THE CAT AFTER SECTION OF THE SACRAL NERVES[1]

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Investigators have found it difficult to agree concerning the type of reflex micturition which develops after section of the sacral nerve roots in the cauda equina as opposed to that occurring after transverse lesions of the spinal cord. Denny-Brown and Robertson (1933) have recently studied several patients of both types and have shown that spontaneous, intermittent micturition may occur after injury of all the sacral nerve roots. A more facile and complete development of automatic micturition, subjected to an interplay of spinal reflexes followed when there was a transection of the spinal cord above the sacral segments.

A study of patients does not give complete information concerning development of automatic micturition under these conditions inasmuch as the behavior of the normal bladder of the individual has not previously been determined. Moreover, the exact extent of the anatomical lesion is seldom known. We have previously reported experimental studies of bladder function freed from parasympathetic control (Langworthy, Reeves and Tauber, 1934). Since that time the preparation of graphic records of vesical activity has brought out further information.

METHOD. Twenty-eight female cats were used in the experiments; they were completely anesthetized with nembutal at the time of the operation, and each time a reading was made. A fine glass catheter was inserted into the bladder through the urethra; it was held in place by the tone of the muscle; and fluid escaped around it when the bladder emptied. By means of a T tube, this catheter was connected with a source of fluid and with a water manometer. Graphic records were made by means of a tambour working with an air column above the fluid of the manometer. The intravesical pressure in centimeters of water was noted upon the tracings. Due to the coefficient of stretch of the rubber, the amplitude of movement of the lever was greatest at a low pressure and decreased rapidly as the pressure rose. The bladder was irrigated with oxycyanide of mercury solution of 1 to 2000 dilution.

[1] This study of the control of the bladder by the central nervous system was supported by a grant from the National Research Council.

The graphs are of two types depending upon the manner in which fluid was introduced into the system. In figure 1, water was added in 5 cc. units; the rises of pressure at equally spaced intervals indicate these additions of fluid. The pressure was then allowed to fall to a resting level. This method tests the response of smooth muscle to stretch, which is a normal stimulus to contraction. In other cases fluid was allowed to flow

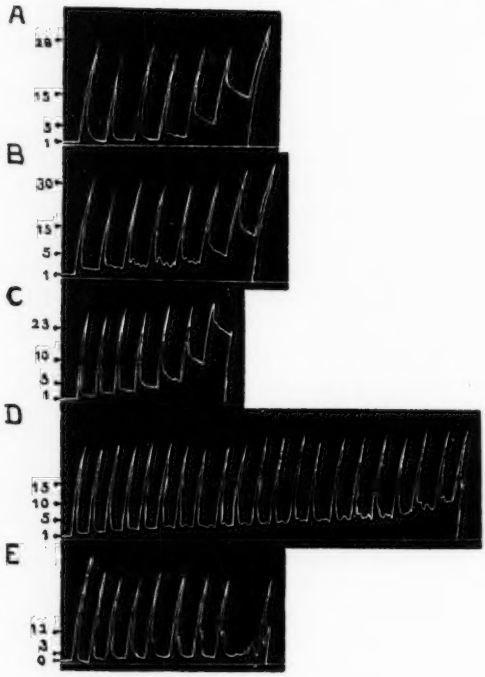

Fig. 1. The vertical lines in the graph indicate the points where 5 cc. units of fluid were added. A, normal reading; B, 3 days after unilateral section of sacral roots; C, 11 days after operation; D, 7 days after section of remaining sacral roots; E, 18 days after second operation.

into the bladder slowly at a constant rate. Figure 2 gives records by this method made on the same animal and the same days as in figure 1. It is difficult to adjust the inflow of fluid and the speed of the drum to a constant rate. Therefore the records do not show an absolute comparison in length depending on the capacity of the bladder. The reflex volume was recorded on the record in each case.

The graphs record the activity of the bladder muscle during filling, and

the intravesical pressure at the moment when the sphincters relax and fluid escapes from the urethra.

EXPERIMENTAL FINDINGS. The results of a typical experiment are shown in figures 1 and 2. Both the dorsal and ventral sacral roots were cut intradurally first upon one side and eleven days later upon the other. Readings were made at periods calculated to show the maximal changes in the bladder and maintain the animal in good health. Recovery from the

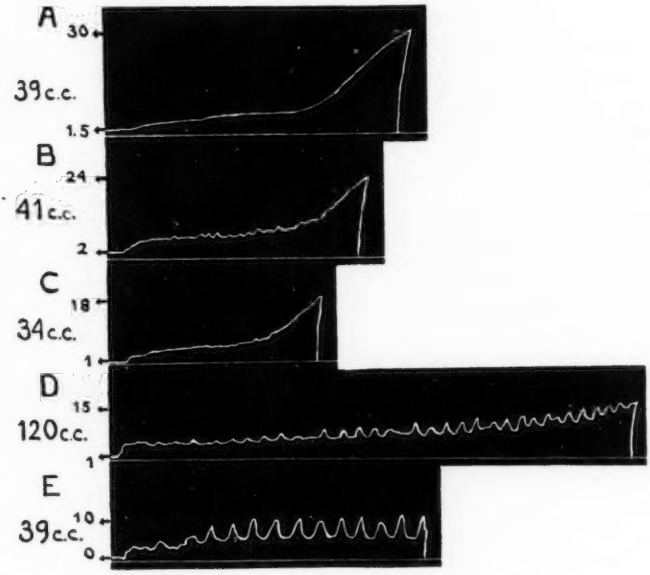

Fig. 2. These readings were made upon the same animal on the same days as those in figure 1. Fluid was allowed to flow slowly and continuously into the bladder. The figures indicate the pressure in the empty bladder and the intravesical pressure at the moment when fluid escaped from the urethra.

operation was uneventful, and no infection was noted either in the operative incision or in the bladder.

Graph A in figure 1 is a record of bladder filling in the normal animal. The pressure was 1 cm. of water in the empty bladder. The resting pressure remained below 5 cm. when the bladder held 20 cc. There was then a progressive rise of pressure until the bladder emptied at a volume of 35 cc. and at an intravesical pressure of 28 cm.

The record in graph B was made 3 days after section of the dorsal and ventral sacral nerve roots of one side. The vesical capacity was increased by 5 cc. The pressure at which the bladder emptied was approximately

the same as in the normal. Small waves of vesical contraction were seen at an intermediate stage of filling. They were of greatest amplitude after the addition of a unit of volume and were stimulated by stretch. As filling became nearly complete these waves disappeared.

The reading shown in graph C was taken 11 days after the first and just before the second operation. Waves of contraction were not as pronounced as in graph B, but they were noticeable after the addition of the fifth unit of fluid. The volume was that of the normal bladder. The emptying pressure of 23 cm. was lower than in either of the preceding records.

Seven days after the second operation completing the section of all the sacral roots, there was an increase in bladder volume to 105 cc. as shown in graph D. Definite waves of vesical contraction in response to stretch appeared when the bladder held 30 cc. and were present until the bladder emptied at an intravesical pressure of 15 cm.

The final tracing E was made 18 days after the second operation and 29 days after the experiment was begun. The emptying pressure was 12 cm., a figure lower than in any of the previous records. When the volume was 25 cc. the pressure did not fall at once to a resting level, but a contraction occurred in response to stretch. The addition of all further increments produced similar responses which raised the pressure to 12 cm. Each of these contractions was associated with the escape of a small quantity of fluid from the urethra. The volume of this bladder should be considered 30 cc. although the filling was continued to 45 cc. The sudden addition of a quantity of fluid was responsible for the contraction of the muscle associated with the momentary escape of fluid from the urethra.

The readings shown in figure 2 were made by the method of continuous filling at the same times as those shown in figure 1. They are more accurate for bladder volume and intravesical pressure at the time of escape of fluid. Graph A is the normal reading. No vesical contraction occurred during filling. The pressure was maintained at a low level during the early part of filling and then rose to an emptying pressure of 30 cm. The volume was 39 cc. as opposed to 35 cc. in the previous normal record (graph A, fig. 1).

Graph B, made 3 days after unilateral section of the sacral roots, showed small rhythmical contraction waves at an intermediate stage of filling corresponding to those seen in figure 1, graph B. The waves disappeared as the pressure rose. The pressure at which fluid escaped fell from the normal 30 cm. to 24 cm. The volume was increased to 41 cc.

The reading taken 11 days after the first operation (graph C) showed a vesical capacity of 34 cc. or 5 cc. less than the normal. Contraction waves could just be observed; they were of smaller amplitude than in graph B. The pressure at the time of escape of fluid from the bladder showed a further drop to 18 cm.

Seven days after completing section of all the sacral nerve roots, the volume was 120 cc. (graph D). Fluid escaped at a pressure of 15 cm. Rhythmical contraction waves were seen throughout the period of filling. They increased in amplitude, and fluid finally escaped at the height of one of the waves. There was a gradual rise in the base line of intravesical pressure as filling progressed, and on this base line the waves were superimposed.

The rhythmical waves were of greater amplitude 18 days after the final operation (graph E). With 39 cc. in the bladder, fluid escaped at the height of one of these waves at a pressure of 10 cm. of water. The volume at this time was the same as in the normal animal.

Section of the sacral nerve roots produces two changes in the graphic record, both of which are shown clearly in figure 2. First, there is a decrease in the height of intravesical pressure at the time when the fluid escapes from the urethra. This suggests that the vesical sphincters relax at a lower pressure. Second, contraction waves of regular amplitude and periodicity appear in the record; they show no tendency to summation. When these waves carry the intravesical pressure to a level at which the sphincters relax, a small quantity of fluid escapes at the height of each wave. The two factors of amplitude of the waves and fall of height of the intravesical pressure at which fluid escapes are related to the eventual bladder volume and the efficiency of periodic micturition. They show considerable variability in different preparations.

The records obtained from another cat are shown in figure 3 as a further example of a marked development of the rhythmical contraction waves. Graph A is the normal curve. The volume was 40 cc. and the intravesical pressure at the time fluid escaped was 35 cm. of water. Record B was made 4 days after section of the sacral roots on the right side. The vesical volume was increased to 50 cc. A few small contraction waves were seen throughout the record. With the sacral roots intact on one side, a fusion of contraction waves was possible. This was seen at the end of the record where a steady rise of pressure caused fluid to escape from the urethra at 16 cm.

The volume of the bladder increased greatly after section of all the sacral roots; and 8 days after the operation, the bladder held 170 cc. or over four times the normal amount (graph C). Contraction waves of small amplitude were seen throughout the record. Certain of these were stretch responses after the addition of fluid. Fluid escaped at a pressure of 25 cm. of water.

D and E were records made 18 days after the second operation or 26 days after the experiment was begun. Graph D shows filling by the addition of 5 cc. units of fluid whereas graph E shows continuous filling. In D large waves of regular amplitude and periodicity occurred after each unit of

fluid was added. Responses of the muscle to stretch after the second, third, and fourth units were added produced a momentary escape of fluid and denoted an emptying pressure in the vicinity of 15 cm. The volume was definitely less than 20 cc. and filling was stopped. Many large waves are seen in graph E before filling was begun. As fluid entered the bladder the waves increased in amplitude, and fluid escaped when the pressure exceeded 15 to 16 cm. Fluid entered the bladder faster than it escaped. The base line of pressure rose till it exceeded 15 cm. and escape of fluid was almost continuous. The reading was stopped at this point.

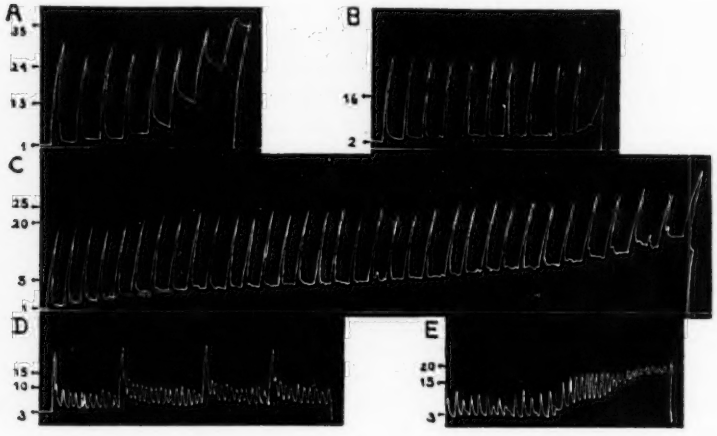

Fig. 3. Readings from another preparation. A, normal reading; B, 4 days after unilateral section of sacral roots; C, 8 days after section of remaining sacral roots; D and E, 18 days after second operation.

Discussion. Section of the sacral nerve roots on one or both sides produced temporary enlargement of the bladder. This was not marked after cutting the roots unilaterally, and within ten days the vesical volume returned to normal or slightly less than normal. The enlargement was greater when all the roots were cut but again it was seldom permanent. Some degree of periodic micturition developed, although the degree of its efficiency varied in different animals. It was with the onset of periodic micturition that the vesical volume was decreased. The onset of periodic micturition was dependent upon two factors,—the development of waves of vesical contraction, and the escape of fluid from the bladder at an intravesical pressure far below that of the normal individual.

We have previously studied the effect upon micturition of section of the posterior sacral roots alone (Dees and Langworthy, 1935). Under these

conditions the vesical volume became large and remained large throughout the life of the animal. The condition was similar to that observed in cases of tabes dorsalis. No waves of vesical contraction appeared in the graphic records, and the intravesical pressure at which fluid escaped from the urethra remained at a normal height. If the abdominal sympathetic trunks were then removed from these same animals, rhythmic waves of vesical pressure appeared in the records, and fluid escaped from the urethra at a lower intravesical pressure. The cats subsequently developed some degree of periodic micturition.

In the present experiments the development of the waves of vesical contraction could not be dependent upon section of the posterior sacral roots as the above experiments show. It must rather be due to severing the anterior roots which contain the parasympathetic fibers innervating the bladder. The rhythmical waves represent inherent activity of the vesical muscle and intrinsic nerve plexus freed from central control. It is unlikely that these waves are the result of contraction of smooth muscle without nervous control, inasmuch as a large group of the smooth muscle fibers contract at once. This postulates a possible control through the intrinsic nerve plexus. Schwartz (1934) has recently given proof of reflex activity controlled by the peripheral portion of the sympathetic nervous system.

The waves are unusual in their periodicity and in their rather constant amplitude. They show no tendency to summation to produce sustained contraction. Summation occurs through the influence of reflex arcs acting through the sacral portion of the cord. After transection of the cord in the lower lumbar region, summation of contraction waves may be produced by stimulation of cutaneous areas supplied by the isolated portion of the cord. In the experiments, fluid escaped from the bladder only at the height of the contraction waves. Therefore the small waves occurring after section of the sacral roots discharged only a small amount of urine at one time. The bladder muscle responded to stretch stimuli. This often produced a contraction of unusual strength.

Denny-Brown and Robertson (1933) observed a degree of summation of contraction waves in two of their three patients with injury of the cauda equina. The third showed rhythmical waves of the type described here. They believed the lack of summation in the third case was dependent on severe cystitis which injured the intrinsic nerve supply. In view of the experiments it is possible that the injury of the sacral nerves was incomplete in the first two cases.

The relation between vesical contraction and sphincter relaxation is still poorly understood. In the experiments, we have attempted only to measure the intravesical pressure at the moment when fluid escaped from the urethra. It is possible that this determination gives very exact information concerning the activity of the vesical sphincters. The most reliable

readings were made by allowing fluid to flow continuously into the bladder. No fluid escaped from the bladder during the introduction of units of fluid even though the pressure rose to a very high level.

It will be remembered that cutting the posterior sacral roots alone did not change the intravesical pressure at the time fluid escaped. On the other hand section of the both dorsal and ventral sacral roots first unilaterally and then bilaterally caused a progressive decrease in the emptying pressure (fig. 2). This was related both to the length of time following the operation and the extent of the nerve injury. There are only two exceptions noted in the graphs (B in fig. 1 and C in fig. 3). These two records were made soon after section of the nerve roots in the period of shock before any marked rhythmic contractions of the muscle had become established.

Two possible explanations for the lowered intravesical pressure at the moment of initiation of periodic micturition may be considered; one or both of them may be important. Section of all the motor sacral roots may have produced relaxation of the external sphincter and also general wasting of the perineal muscles allowing fluid to escape from the bladder more easily. The development of the rhythmical contraction waves may in itself be responsible for the lowered emptying pressure.

We agree with the findings of Denny-Brown and Robertson (1933), that a contraction of the vesical musculature always precedes relaxation of the vesical sphincters. There is a degree of reciprocal activity between the vesical muscle and the sphincter. Sphincter relaxation occurs at a lower pressure when rhythmical contraction waves are developed. A glance at the records of bladder filling in the normal cats shows that no contraction waves occurred during the process of filling. It was only after section of the nerve roots and concurrent with the development of rhythmical waves that the emptying pressure fell. There is a similar fall coincident with the development of contraction waves after transection of the spinal cord in the thoracic and lumbar region. In this case the internal pudendal nerves are uninjured. It is probable then that the fall of intravesical pressure at the time when fluid escapes from the urethra is dependent upon the development of vesical contractions. The strength of the waves and the fall of the emptying pressure determine the subsequent decrease of vesical volume and the efficiency of periodic micturition.

The period of vesical enlargement following section of the sacral roots may be considered a manifestation of "shock." Later the inherent rhythm of contraction of the muscle appears. There is no longer overflow incontinence, and periodic micturition is re-established. This is a type of automatic micturition of a low degree of efficiency.

We have used the principles set forth here in studies of patients with vesical disturbances due to spina bifida (Langworthy and Dees, 1936). In some cases the posterior and in others the anterior sacral roots are

predominantly involved. Graphic records show a large vesical volume, low intravesical pressures, and absence of vesical contraction with lesions limited chiefly to the sensory roots. A small bladder with rhythmic contraction waves and escape of fluid at low intravesical pressure suggests an involvement of the motor roots.

SUMMARY

The motor and sensory sacral nerve roots were cut in cats, first upon one side and afterwards also on the other side. Graphic records of vesical activity were obtained from the normal animals and at suitable periods after the operations. There was a slight increase in vesical volume in the first few days after section of the roots unilaterally. The volume then returned to normal or slightly less than normal. A greater enlargement of the bladder followed section of all the sacral roots. Later automatic micturition became established; its efficiency varied in different cases. The time of onset and the efficiency of micturition were related to the development of rhythmical contraction waves in the vesical muscle and to a lowering of the intravesical pressure at the time fluid escaped from the urethra. The contraction waves showed no summation; they were increased in amplitude in response to stretch stimuli. With the onset of periodic micturition, the volume of the bladder was decreased to less than normal.

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AN EXPERIMENTAL STUDY OF MICTURITION RELEASED FROM CEREBRAL CONTROL[1]

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The authors have attempted to prove experimentally that removal of the cerebral motor cortices of cats produces permanent changes in vesical function similar to those reported in acute preparations (Langworthy and Kolb, 1933). The experiments were difficult of execution, and gave somewhat inconstant results. Later study of patients with cortico-spinal tract injuries not only confirmed the previous findings, but also illuminated the experimental difficulties (Lewis, Langworthy and Dees, 1935).

It is advantageous in studying the smooth muscle of the bladder wall to keep in mind Hughlings Jackson's concept of levels of function in the nervous system. There are peripheral sympathetic and parasympathetic nerve fibers innervating the bladder; the functions of these two systems have been discussed in a previous paper (Langworthy, Reeves and Tauber, 1934). In the lumbo-sacral portion of the spinal cord, segmental and intersegmental reflex arcs coördinate and control vesical activity. A further control of tone in the bladder musculature is mediated by reflex arcs extending as high as the upper end of the mid-brain. The position of this mechanism has been outlined by electrical stimulation, and the effects of its release and removal have been studied (Langworthy and Kolb, 1933 and 1935a). Finally, the highest control of micturition lies in the cerebral cortex. Through the cortical control it is possible to postpone micturition by lowering the intravesical pressure or to initiate the act of bladder emptying. The last two levels of vesical control are of the nature of suprasegmental reflex arcs. They cannot be considered to exert their influence through either sympathetic or parasympathetic channels until this can be proved experimentally.

This study of the cerebral cortex in relation to micturition in animals must deal exclusively with the motor side of the reflex arc. Sensory impulses reach the cerebral cortex giving information concerning distention, the painful effects of overdistention, or bladder irritation.

There is a direct cortical control of the smooth muscle of the bladder wall

[1] This study of the control of the bladder by the central nervous system was supported by a grant from the National Research Council.

(Langworthy and Kolb, 1935a). Aside from voluntary closure of the external sphincter which contains striated muscle, the vesical sphincters appear to have little representation in the cerebral cortex (Denny-Brown and Robertson, 1933a, b).

Hughlings Jackson divided the abnormalities of striated muscle after injury of the nervous system into four groups. These may also be applied in the case of the bladder muscle. The divisions are irritation, "shock," loss of function, and release of function. The instances of bladder incontinence during convulsive seizures may be considered as due to irritation. We have not studied "shock" in the vesical muscle of patients with unilateral or bilateral injury of the cortico-spinal tracts, but it is known to occur. "Shock" as well as loss of function and release of function will be considered in the experimental preparations.

It is to be expected that the abnormalities in bladder function after removal of the cerebral motor cortices in cats will not be as marked or constant as after similar injuries in man. This is true of abnormalities of striated muscle function (Langworthy, 1932). The patients were not anesthetized during the study of vesical activity; the animals were in every case placed under deep anesthesia.

METHOD. Female cats have been utilized in the work, and data have been collected from 51 individuals. There were technical difficulties in carrying through the experiments due to the behavior changes which follow removal of the second motor cortex, and the large number of readings which must be made under anesthesia in the case of one experimental animal. The behavior changes suggested the syndrome of pseudo-bulbar palsy observed in man after bilateral injury of the cortico-spinal tracts (Langworthy and Kolb, 1935b). The cats had difficulty in eating and drinking for the first few days following the operation; later they had abnormally large appetites. They showed a great increase in activity and walked continuously. At first it was impossible for them to turn aside from a straight line of march to avoid objects or escape from corners.

The reflex capacity of the bladder of a normal anesthetized cat shows little variability. The procedure of making a bladder reading causes some irritation of the bladder and urethra, but the reflex capacity becomes constant for the individual if the readings are made every two days over a period of time.

The cats were anesthetized with nembutal before operations and before bladder readings were made. The nembutal kept the animals quiet and comfortable for several hours after removal of the motor cortex.

To make the readings, a fine glass catheter was passed into the bladder through the urethra. The catheter was not tied into the bladder, but was held in position by the tone of the sphincters. With the onset of micturition, fluid was able to escape freely about the catheter which then became

loose in the urethra. The catheter was connected with a three-way tube to which were attached a water manometer of 3 mm. bore and a burette graduated to one-tenth of a cubic centimeter. By means of the burette, 1–2000 oxycyanide of mercury solution, warmed to body temperature, was

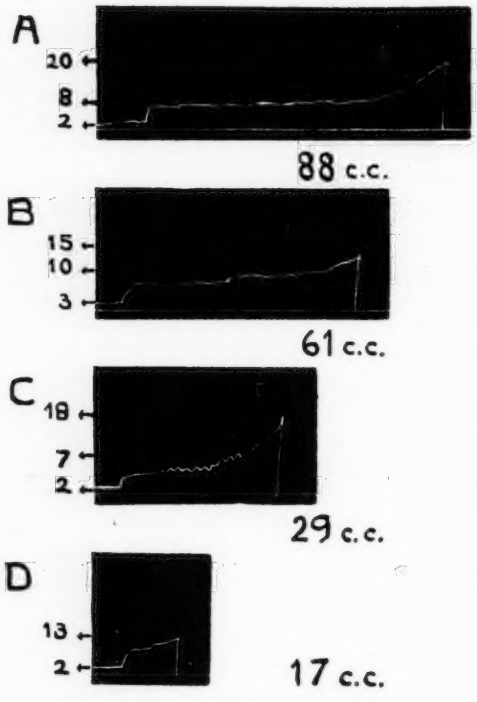

Fig. 1

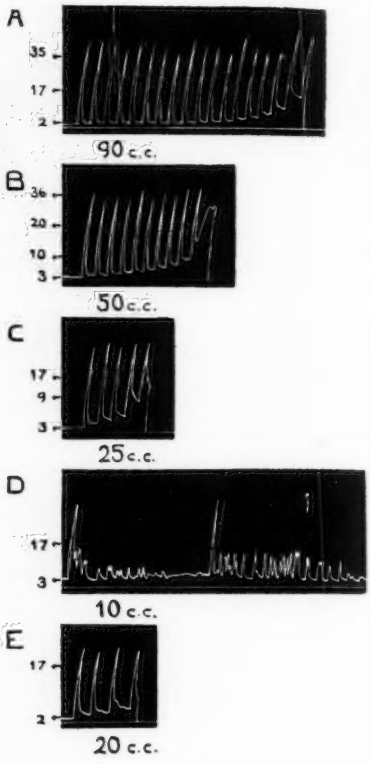

Fig. 2

Fig. 1. Readings following removal of the cerebral motor cortices. A, normal; B, immediately after extirpation left motor cortex; C, three days later; D, six days after extirpation second motor cortex.

Fig. 2. Readings following removal of the cerebral motor cortices. A, normal; B, directly after removal left motor cortex; C, nine days later; D, directly after extirpation second motor cortex; E, 10 days later.

allowed to flow slowly into the bladder. Graphic records were made by attaching a tambour to the top of the manometer tube. The initial pressure reading was made with the bladder empty. Then equal quantities of fluid of 5 cc. were introduced, and the pressure was read when it reached

a resting level. Other records were made by allowing fluid to run slowly and continuously into the bladder at the rate of 5 cc. a minute.

With aseptic precautions the cerebral motor cortex was exposed upon one side. The entire anterior pole of the cerebral cortex, including the gyrus proreus and the underlying olfactory cortex, was removed in a single slice. This cut did not expose the lateral ventricle or the head of the caudate nucleus. At a later time a similar operation was performed upon the second side. Bladder determinations were made, directly after the operations, and at suitable intervals thereafter.

EXPERIMENTAL FINDINGS. The experimental findings will be reported as a description of a series of readings made upon 3 cats (figs. 1 to 3). In the first demonstration the fluid was allowed to flow slowly but continuously into the bladder during the vesical filling (fig. 1). The normal bladder (A) held 88 cc. of fluid; water escaped from the urethra at a pressure of 20 cm. The pressure in the empty bladder was 2 cm. of water and remained below 8 cm. until filling was nearly complete. The left cerebral cortex was then removed and the second reading made immediately thereafter (B). The bladder then held 61 cc. and fluid escaped at a pressure of 15 cm. Eight days following removal of the left motor cortex (C) the bladder capacity was 29 cc.; the intravesical pressure at the time fluid escaped was 18 cm. On that day the second or right motor cortex was removed. No further reading was made for 6 days. Then the bladder (D) held only 17 cc., and fluid escaped around the catheter at a pressure of 13 cm.

The bladder volume decreased after removal of one motor cortex from 88 to 29 cc., and after removal of the second motor cortex to 17 cc. The intravesical pressure at which fluid escaped from the urethra was also lowered from 20 cm. in the normal to 18 cm. after removal of one motor cortex and to 13 cm. after removal of the second motor cortex.

The second series of readings was made from another preparation; here the fluid was added in equal quantities of 5 cc. (fig. 2). The vertical lines upon the graphs indicate the points where units of fluid were introduced into the system. The normal bladder (A) held 90 cc. and fluid escaped from the urethra at a pressure of 35 cm. Intravesical pressure remained low during filling. The second reading (B) was made directly after removal of the left motor cortex. The bladder held 50 cc. and fluid escaped from the urethra at 36 cm. of pressure. The third reading (C) was made 9 days after the left cerebral motor cortex was cut away. The capacity of the bladder was 25 cc. and fluid escaped at a pressure of 17 cm.

The fourth reading (D) was made immediately after removal of the second or right cerebral motor cortex and demonstrated activity which we believe is dependent on "shock." After the addition of the first 5 cc. of fluid, rhythmical waves of vesical contraction occurred. The pressure was

slow in falling to a resting level. The waves were marked immediately after fluid was introduced and became less pronounced during the period that the volume remained constant. When another quantity of fluid was added, the waves became of greater amplitude. They raised the pressure to 17 cm. A quantity of fluid escaped from the bladder at the peak of each wave. For this reason the filling was stopped.

The final reading (E) was made 10 days after the removal of the second cortex. The reflex volume of the bladder then was 20 cc., and fluid escaped from the urethra at 17 cm. of water pressure.

This cat had a normal bladder volume of 90 cc. The volume was decreased to 25 cc. after removal of the first and to 20 cc. after removal of the second cerebral motor cortex. In the normal animal fluid escaped from

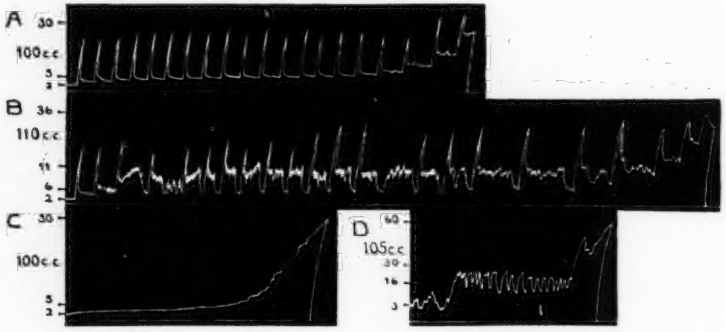

Fig. 3. Demonstration of "shock" immediately following removal of the left cortex. Graphs A and C were normal readings. Graphs B and D were made directly after the operation.

the urethra at an intravesical pressure of 35 cm. Following removal of the motor cortices this pressure fell to 17 cm.

If the animals are studied for a considerable period of time, the bladder does not hold its initial small volume after removal either of one or both cerebral motor cortices. The volume tends to increase although never to approach the normal. The animal described above was studied again 4 months after the second motor cortex was extirpated. The volume had increased to 55 cc. The intravesical pressure when fluid escaped was below 15 cm. of water.

Figure 3 gives another example of "shock" following extirpation of the left cortex. A and C are readings made from the normal cat, and B and D immediately after removal of the left cerebral cortex. In graphs C and D fluid entered the bladder continuously. The normal bladder held 100 cc., and the intravesical pressure was 30 cm. at the time when fluid escaped.

After the left cerebral cortex was extirpated, a change in vesical activity was apparent (graph B). The pressure did not fall at once to a resting level after the introduction of fluid. The increase of volume produced a stretch response of the muscle. Rhythmical waves of contraction appeared which are seen more clearly in graph D. The volume of the bladder was slightly increased; the intravesical pressure at the moment of escape of fluid was higher than before operation. When the next reading (not shown) was made three days after the operation, the volume was 35 cc. and the record no longer showed the rhythmical waves of contraction.

Decerebration of the cats several days after the second motor cortex was removed produced no further decrease in the volume of the bladder.

The cats lost their former habits of micturition following removal of the second cerebral motor cortex. When the bladder was full they became restless and moved about more actively. Micturition and defecation no longer were performed in special, suitable places but wherever the animal happened to be at the time. With the beginning of the flow of urine the cat assumed a characteristic posture.

Discussion. Men with bilateral injury of the cortico-spinal tracts complain of urgency, frequency, and incontinence. The resting pressure in the empty bladder and during filling is usually somewhat higher than normal. The vesical capacity is small. The rise of pressure at the end of filling is precipitous and greater than normal. The muscle responds actively to stretch stimuli. At the time of catheterization the external sphincter offers considerable resistance to the passage of a catheter.

The bladder volume of the cats was decreased after removal of the cerebral motor cortex on one side. A further reduction of volume followed extirpation of the second cerebral cortex. If decerebration was done at a later date no further reduction of vesical volume occurred. The intravesical pressure at which fluid escaped from the bladder was decreased after removal of one motor cortex, and was further reduced after removal of the second cortex. This appears to be dependent upon increased strength of contraction of the vesical muscle. In man the increased tone of the striated muscle of the external sphincter is comparable to the increased tone of the muscle in the extremities. Inasmuch as this hypertonus following removal of the cerebral motor cortex is not marked in cats, the external sphincter did not show resistance to the catheter. For the same reason, the intravesical pressure at the time the bladder emptied was not elevated as it is in man. After removal of the cerebral motor cortex of the cat, the bladder loses to some extent its ability to accommodate and hold increasing quantities of fluid at approximately the same pressures (fig. 2, graphs A and C).

The increased response of the muscle to stretch was not demonstrated well in the cats. This may be due in part to the anesthetic always used in the experimental work. Again, the stretch reflex in striated muscle is not

as marked after injury of the motor cortices as it is in man. During the period of "shock" which followed the operation, an increased response of the muscle to stretch could often be observed.

Following an injury of the cortico-spinal fibers in man, a period of "shock" intervenes. The bladder becomes distended and retention develops. Catheterization is necessary. This "shock" of the muscle of the bladder of cats has been demonstrated in the records. Rhythmical contractions are a property of the vesical muscle itself and are brought out characteristically following section of the parasympathetic nerves to the bladder. During the period of "shock" these rhythmical waves appear.

The decrease of reflex volume of the bladder may be interpreted as a release of function. The tonic mechanism in the mid-brain becomes hyperactive when released from cortical control. This is well demonstrated in man as an increased activity of the stretch reflex. Loss of function is demonstrated in man as an inability to control the waves of vesical contraction and so postpone micturition. The cats no longer retain the normal habits of micturition.

SUMMARY

The effect of removal of the cerebral motor cortices upon the function of micturition in cats was studied. The vesical capacity was reduced after extirpation of one motor cortex, and a further decrease in capacity followed removal of the second cortex. Decerebration at a later date produced no further changes in vesical volume. The intravesical pressure at the time fluid escaped from the urethra was reduced with extirpation of the motor cortices. The bladder lost to some extent its capacity to accommodate increasing quantities of fluid at low pressures. "Shock" in the smooth muscle of the bladder could often be demonstrated immediately after the operation.

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THE INFLUENCE OF IRRADIATED ERGOSTEROL AND PARATHYROID EXTRACT UPON THE RATE OF DISAPPEARANCE OF INTRAVENOUSLY INJECTED CALCIUM CHLORIDE

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It is generally recognized that intravenously injected calcium disappears rapidly from the blood stream of normal animals, a return to the initial level occurring three or four hours after injection (1–4). The immediate fate of this rapidly disappearing calcium is unknown, but there is some evidence that the bulk of it is not excreted immediately and that the skeleton may serve at least as a temporary repository (2, 5). As pointed out by Taylor (5), injection of calcium sufficient to theoretically raise the blood serum level to seven or eight times normal actually produces an elevation which is approximately only twice normal even as soon as five minutes after injection. According to Greville (4), the rate of fall of the serum calcium at any period longer than five minutes after injection is proportional to the excess of serum calcium above the final constant level.

It was our desire to determine whether or not parathyroid extract or irradiated ergosterol influenced the rate of removal of calcium from the blood stream. To accomplish this end it was also necessary to establish the uniformity of the rate of removal of injected calcium from the circulating blood of normal dogs.

EXPERIMENTAL. Healthy adult dogs were used ranging in weight from 12 to 20 kgm. Their stock diet consisted of cooked ground meat, bone meal and white bread. During the week of irradiated ergosterol administration the dogs received a diet of cooked corn-meal and white bread, a diet which has been shown (6) to have a low calcium content. All injections of calcium were carried out 24 hours after the last feeding.

All serum calcium determinations were made in duplicate by the Clark-Collip (7) modification of the Kramer-Tisdall (8) method. The calcium solution used in these experiments contained 10 mgm. of calcium in 1 cc. This solution was prepared by diluting a more concentrated solution, the calcium content of which had been checked by analysis.

The uniformity of the rate of removal of intravenously injected calcium from

the blood stream of normal dogs: Six normal dogs were injected twice, the same dose being used for each injection; the second injection was given one week after the first. Four of the dogs received 15 mgm. of calcium per kilogram while the other two received 10 mgm. per kilogram. Blood samples were taken just before injection, 5 minutes afterwards, and at hourly intervals thereafter for the following 4 hours. The results (table 1)

TABLE 1

The uniformity of the rate of disappearance of injected calcium from the blood stream of normal dogs

	FASTING LEVEL*	SERUM CALCIUM INCREASE				
		5 minutes	1 hour	2 hours	3 hours	4 hours
Dog 1†						
1st injection	10.1	6.4	1.5	0.6	0.2	0.4
2nd injection	10.1	6.4	1.7	0.5	0.1	−0.1
Dog 2†						
1st injection	10.8	4.6	1.2	0.7	0.3	0.1
2nd injection	10.8	4.3	1.6	0.6	0.3	0.2
Dog 3						
1st injection	10.8	7.9	2.3	1.5	0.8	0.4
2nd injection	11.0	7.5	2.9	2.7	2.3	0.7
Dog 4						
1st injection	11.6	8.2	4.1	1.9	0.7	0.1
2nd injection	11.5	9.7	2.8	1.4	0.8	−0.3
Dog 5						
1st injection	10.2	7.6	3.6	2.6	2.0	1.3
2nd injection	10.8	7.0	4.3	2.8	2.2	0.7
Dog 6						
1st injection	10.5	6.8	4.1	3.3	2.8	2.3
2nd injection	10.8	8.2	4.2	3.3	3.1	2.0
Average values						
1st injection	10.6	6.9	2.8	1.8	1.1	0.7
2nd injection	10.8	7.2	2.9	1.9	1.3	0.6

* All calcium values are expressed in terms of milligrams per 100 cc. of serum.

† These dogs were given 10 mgm. calcium per kilogram body weight, the other four received 15 mgm. calcium per kilogram body weight.

show practically the same rate of disappearance in any given dog on the two tests made a week apart, hence the rate of removal is evidently constant for normal dogs.

The influence of parathyroid extract upon the rate of removal of intravenously injected calcium from the blood stream of dogs: The normal rate of calcium disappearance was determined in 8 more dogs, none of which had been used in the preceding series. The dose of calcium was 10 mgm. per

kilogram in this and the subsequent experiments described in this paper. The normal values for these dogs (table 2A) are quite similar to those recorded in table 1. Seven of these dogs were given 12 units per kilogram of parathyroid extract[1] subcutaneously 18 hours before calcium injection.

TABLE 2

Rate of disappearance of intravenously injected calcium

	DOG NUMBER	FASTING LEVEL*		SERUM CALCIUM INCREASE			
		2 hours before injection	Just before injection	2 hours	4 hours	6 hours	8 hours
A. Normal	7		10.7	1.3	0.3	0.2	
	8		10.7	1.1	0.4	0.3	
	9		10.4	2.7	1.4	0.6	
	10		11.6	0.6	0.0	0.0	
	11		10.7	1.3	0.3	0.2	
	12		10.9	2.1	1.3	0.6	
	13		10.8	1.8	0.6	0.1	
	14		10.3	2.0	0.8	0.3	
Averages			10.8	1.6	0.64	0.3	
B. After parathyroid extract	7	13.4	13.7	1.7	1.3	0.6	0.2
	8	13.5	13.2	1.8	1.1	0.3	0.0
	9	17.1	17.6	2.0	1.9	1.8	1.2
	11	13.4	13.7	1.9	0.9	0.9	0.6
	12	14.0	13.6	3.8	3.7	4.1	3.6
	13	13.3	13.7	1.9	1.4	1.2	0.8
	14	13.0	12.9	2.7	2.4	2.3	2.0
Averages		14.0	14.1	2.25	1.8	1.6	1.2
C. After irradiated ergosterol	7		11.2	1.6	0.9	0.6	0.4
	9		15.3	2.7	2.3	1.0	1.0
	10		16.2	1.8	1.2	1.3	
	11		11.4	0.8	1.0	0.9	0.9
	12		15.7	2.7	1.6	1.4	1.1
	13		13.6	1.7	1.5	1.3	1.3
Averages			13.9	1.9	1.4	1.1	0.9

* All calcium values are expressed in terms of milligrams per 100 cc. of serum.

The serum calcium was determined 2 hours before and just before calcium injection as well as 2, 4, 6 and 8 hours after injection. The two calcium determinations before injection were intended to indicate the rate and

[1] We are indebted to Eli Lilly for providing us with the parathyroid extract used in these experiments.

direction of change of the serum calcium as a result of the parathyroid extract injection. These results (table 2B) show a prolongation of the serum calcium elevation in six of the seven dogs.

The influence of irradiated ergosterol upon the rate of removal of intravenously injected calcium from the blood stream of dogs: After two weeks' rest five of these animals and one dog not used for the parathyroid experiments (no. 10) were placed on a low mineral diet and were given 2 doses each of 5,000X Viosterol;[2] the dosage in every case but that of no. 10 was 0.10 cc. per kilogram body weight (no. 10 received 0.12 cc.). Seven days later calcium injections were made, blood samples being taken before injection, and 2, 4, 6 and 8 hours afterwards. The results of this experiment (table 2C) show a prolongation of the hypercalcemia as compared to the normal rate of calcium removal and demonstrate a similarity in the influence of parathyroid extract and irradiated ergosterol upon the rate of disappearance of calcium from the blood stream.

Discussion. The oral administration of calcium in conjunction with the injection of parathyroid extract has been shown (11) to cause a marked elevation of the serum calcium in dogs. Hamilton and Schwartz (12) have proposed a method for the determination of the parathyroid hormone in blood which is based upon the premise that the serum calcium elevation produced by oral calcium administration is greater in the presence of an increased amount of parathyroid hormone in the blood stream. Irradiated ergosterol has also been shown to influence the serum response to ingested phosphates and calcium in rabbits (13) and dogs (14). Our results demonstrate that at least a part of the hypercalcemia observed upon calcium administration to animals treated with irradiated ergosterol or parathyroid extract may be due to a decreased rate of calcium removal from the blood stream, a factor that must be considered in experiments on substances which elevate the serum calcium.

The dogs reported in this paper did not possess the same preinjection concentration of serum calcium after parathyroid extract or irradiated ergosterol as when their normal rate of calcium removal was determined. This fact makes it impossible to decide whether the retarded rate of removal is due to anything more specific than the initial elevation of the serum calcium. Probably the tissues are more nearly saturated with calcium at the higher serum level, and their capacity to combine with serum calcium is correspondingly reduced.

SUMMARY

The uniformity of the rate of removal of injected calcium chloride has been demonstrated in six normal dogs. It has also been shown that the administration of sufficient parathyroid extract or irradiated ergosterol to

[2] Furnished us through the courtesy of Mead Johnson and Company.

produce a fasting hypercalcemia will cause a decreased rate of removal of calcium from the blood stream of dogs.

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THE CLEARANCE OF HEXAMETHENAMINE IN THE DOG

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Hexamethenamine, when administered to man by mouth in relatively small doses, appears rapidly in the urine in appreciable quantities. Since it is a biologically inert, nitrogen-containing substance of a molecular weight of the same order of magnitude as creatinine, we have examined its excretion in the dog, relative to the simultaneous excretion of creatinine and xylose, both before and after the administration of phlorizin.

The therapeutic use of hexamethenamine is based on its hydrolysis in the acid urine of the tubules and lower urinary tract to formaldehyde. In the present experiments this decomposition was avoided by rendering the urine strongly alkaline. All urines were tested and shown to be alkaline, and numerous qualitative tests established the uniform absence of free formaldehyde, so that we may assert that our observations deal with the excretion of hexamethenamine, uncomplicated by any decomposition.

Female dogs were used, the bladder being carefully emptied by catheter. The dogs were maintained on a mixed diet to which 5 grams of sodium bicarbonate were added each day. Creatinine was administered subcutaneously in doses of 500 mgm. per kilo in 10 per cent solution and hexamethenamine (Formin, Merck) was given intravenously in 15 per cent solution containing 2 per cent sodium bicarbonate. This latter solution was not sterilized because of the danger of heat decomposition. No reactions were observed from doses of 70 grams administered intravenously to dogs weighing from 15 to 20 kgm. There was no bladder irritation, and at no time was blood observed in the urine.

In figure 1 fifty-three hexamethenamine/creatinine clearance ratios, obtained in experiments on three dogs are charted against the concentration of hexamethenamine in the plasma. The clearance ratio is independent of the plasma concentration of hexamethenamine between 56 and 561 mgm. per cent. Since Shannon (1935) has shown that the creatinine clearance is independent of plasma concentration in the dog, it follows from the above that the hexamethenamine clearance is likewise independent of its plasma concentration.

The average of the 53 clearance ratios is 0.761, with maximum variations of 0.70 and 0.82.

Since this value is approximately that obtained in numerous other investigations in which the simultaneous xylose and creatinine clearances were compared (Shannon, Jolliffe and Smith, 1932; Pitts, 1934; etc.), the hexamethenamine clearance was compared with the xylose clearance. The average hexamethenamine/xylose clearance ratio of 21 comparisons on three dogs is 0.986 with maximum variations of 0.94 and 1.06. The data for experiment 8, given in table 1, are typical of this series of experiments.

Three series of observations were then made comparing the excretion of the three substances before and after the administration of phlorizin, of which experiment 13, table 1, is an example. The three experiments yielded essentially the same results. It may be seen that all clearances are depressed to some extent by phlorizin, as has been noted by investigators using this drug (Shannon, 1935), the xylose clearance being depressed

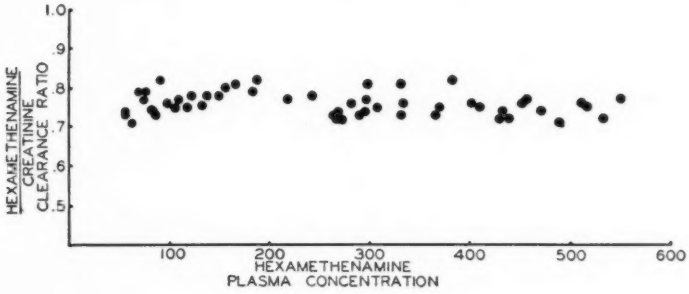

Fig. 1

the least. The hexamethenamine/creatinine clearance ratio is not appreciably affected, while the xylose/creatinine clearance ratio rises toward unity and the hexamethenamine/xylose clearance ratio falls. The averages of all our observations are summarized in table 2.

Discussion. If creatinine is accepted as a measure of glomerular filtration in the dog on the basis of the arguments advanced by Shannon and Smith (1935), it follows that hexamethenamine is reabsorbed from the glomerular filtrate to an extent amounting to some 25 per cent of the amount filtered, a degree of reabsorption essentially identical to the reabsorption of xylose. The percentage of reabsorption of hexamethenamine is independent of plasma concentration over the wide range from 56 to 561 mgm. per cent. The reabsorption of xylose, though never studied over as extensive a range, is also independent of its plasma concentration, and it might be assumed that the reabsorbing mechanism is the same for

TABLE 1

Comparison of hexamethenamine, creatinine and xylose clearances in the normal and phlorizinized dog

EXPERIMENT NUMBER	TOTAL CONCURRENT TIME	URINE FLOW PER MINUTE	PLASMA			CLEARANCE			CLEARANCE RATIOS		
			Hexamethenamine	Creatinine	Xylose	Hexamethenamine	Creatinine	Xylose	Hexamethenamine / Xylose	Hexamethenamine / Creatinine	Xylose / Creatinine
					Dog 2						
	minutes	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc. per minute*	*cc. per minute*	*cc. per minute*			
8	14	8.36	458	35.1	110	64.1	83.4	63.8	1.00	0.77	0.77
	28	7.43	411	34.1	117	63.3	84.3	63.5	1.00	0.75	0.75
	43.5	6.71	371	33.0	117	64.2	85.8	65.1	0.99	0.75	0.76
	59	6.46	335	31.4	118	67.5	89.3	69.6	0.97	0.76	0.78
	72.5	5.92	309	29.6	117	65.2	87.0	68.8	0.95	0.75	0.79
	87.5	5.46	291	27.7	115	63.2	86.8	66.0	0.96	0.73	0.76
									0.98	0.74	0.77
					Dog 1						
11	15	7.87	518	42.6	113	41.2	55.0	41.5	0.99	0.75	0.75
	30	5.73	472	40.6	98.5	41.2	57.2	42.4	0.97	0.72	0.74
	45.5	4.96	431	39.3	93.5	39.6	56.4	40.7	0.97	0.70	0.72
									0.98	0.72	0.74
	48 (60)	355 mgm. phlorizin/kgm. intravenously									
	75	4.33	386	39.3	73.0	28.0	35.4	35.6	0.79	0.79	1.00
	91	4.68	357	35.8	62.7	30.0	37.6	37.0	0.81	0.80	0.98
	107.5	4.12	331	33.4	53.3	34.4	44.0	42.8	0.80	0.78	0.97
									0.80	0.79	0.98

TABLE 2

Average clearance ratios of hexamethenamine, xylose and creatinine in the normal and phlorizinized dog

	HEXAMETHENAMINE / XYLOSE		HEXAMETHENAMINE / CREATININE		XYLOSE / CREATININE	
	Number of periods	Ratio	Number of periods	Ratio	Number of periods	Ratio
Normal dog	21	0.986	53	0.761	21	0.764
Phlorizinized dog	9	0.799	9	0.763	9	0.957

both substances. Evidence has been presented, however, by Shannon (1935) that the reabsorption of xylose is at least partly an active process, since the xylose clearance rises relative to the inulin clearance (or the creatinine clearance in the dog) after the administration of phlorizin; consequently if xylose and hexamethenamine were reabsorbed by the same mechanism, the drug would have the same effect on the excretion of the latter substance. On the contrary, our data show that the drug does not have any such effect; the hexamethenamine/creatinine clearance ratio is essentially the same before and after phlorizin, in marked contrast to the rise in the xylose/creatinine clearance ratio. This fact is substantial evidence that the differences between the creatinine and xylose clearances, on the one hand, and the creatinine and hexamethenamine clearances, on the other, are caused by different renal mechanisms. The relatively close agreement of the xylose and hexamethenamine clearances in the normal dog appears to be purely coincidental, indicating that mere identity of two clearances is of doubtful physiological significance.

SUMMARY

1. The hexamethenamine/creatinine clearance ratio in the normal dog is independent of the plasma concentration of hexamethenamine over a range of concentrations from 56 to 561 mgm. per cent, from which it may be inferred that the hexamethenamine clearance itself is independent of the plasma concentration of this substance.

2. The simultaneous clearances of hexamethenamine and xylose are practically identical, both being about 75 per cent of the simultaneous creatinine clearance.

3. The administration of phlorizin depresses the clearances of all three substances, altering the xylose clearance least. The hexamethenamine/creatinine ratio is not changed appreciably by phlorizin, while the xylose/creatinine clearance ratio rises towards 1.0.

Analytical procedures. Analyses of creatinine and hexamethenamine were performed on 1:5 iron filtrates (Steiner, Urban and West, 1932), made with precautions to reduce to a negligible minimum the acid hydrolysis of hexamethenamine. Creatinines were performed as described by Shannon, Jolliffe and Smith (1932). Hexamethenamine was determined in a modification of Romijn's formaldehyde method (1897), by the difference in reducing power of plasma and urine filtrates before and after acid hydrolysis. From 2 to 10 cc. of the 1:5 iron filtrate were pipetted into a 20 × 150 mm. test tube; water was added to make the volume 10 cc. and then 1 cc. of N hydrochloric acid was added. The tubes were capped with glass bulbs and placed in a boiling water bath for ten minutes. This time of hydrolysis is sufficient to hydrolyze completely all hexamethenamine to formaldehyde without any loss by volatilization. The tubes were then removed, cooled, the caps and sides washed down with a small amount of distilled water, and 1 cc. of 3 N sodium hydroxide and 5 cc. of 0.05 N iodine in potassium iodide added. The tubes were allowed to stand one hour, to permit complete oxidation of the liberated formalde-

hyde, and then 1 cc. of 5 N sulfuric acid added and the residual iodine titrated with 0.025 N sodium thiosulfate, using starch as an indicator. If not more than four-fifths of the added iodine is used, the reaction goes to completion. Normal dog plasma contains a blank amounting to about 15 mgm. per cent, calculated as hexamethenamine. About one-half of this is glucose, the rest unknown (but not urea). When xylose or creatinine are present the blank may be increased to from 30 to 70 mgm. per cent, calculated as hexamethenamine. For this reason it is necessary to determine the blank on each plasma and urine sample. This is accomplished by repeating the above analysis, omitting the acid hydrolysis and reversing the order of addition of acid and alkali. Since acid hydrolysis alone does not affect the blank of normal dog plasma, or plasma to which creatinine and xylose are added, the difference in reducing power between the hydrolized and unhydrolyzed specimens may be calculated as hexamethenamine. Unhydrolyzed hexamethenamine has no reducing power by this method. But hexamethenamine is slowly hydrolyzed at room temperature to formaldehyde by solutions of the acidity of the ferric sulfate added in the precipitation method, and to minimize this hydrolysis the filtrates were prepared quickly, never allowing more than 5 minutes to elapse between addition of iron and neutralization with barium carbonate. Under these conditions less than 0.2 per cent of the hexamethenamine present is hydrolyzed, to appear in the blank.

Hexamethenamine, added to plasma and urine in amounts from 40 to 500 mgm. per cent, was recovered with an average error below 2 per cent. Since all analyses, both hydrolysates and blanks, were performed in duplicate, the determination of each hexamethenamine clearance involved eight titrations.

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A FURTHER STUDY OF REFLEX CHANGES OF BLOOD PRESSURE IN COMPLETELY SYMPATHECTOMIZED ANIMALS

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In previous studies from this Laboratory it has been suggested that the dilator fibers in the dorsal spinal roots are probably the efferent paths for the blood-pressure changes which occur in completely sympathectomized cats on central stimulation of the vagus and depressor or somatic afferents (Freeman and Rosenblueth, 1931), on stimulation of the depressor points in the floor of the fourth ventricle (Rosenblueth and Cannon, 1934) and on struggle (Freeman and Rosenblueth, *loc. cit.*).

Bacq, Brouha and Heymans (1934) have disagreed with this interpretation. From their observations on cats they conclude that struggle is attended by a fall of blood pressure because of the metabolites (including CO_2) of muscular activity. The positive carotid sinus reflexes which they recorded they localize exclusively in the splanchnic area and explain as due to activation of constrictor fibers not included in the sympathetic chains. They further report that completely sympathectomized dogs differ markedly from cats in that the former do not show any of the carotid sinus reflexes which are present in the latter.

The present study was undertaken to gather more evidence which might decide between these conflicting interpretations. It was further deemed important to ascertain whether the claim of fundamental differences in the vasomotor reactions of cats and dogs could be confirmed.

METHOD. Cats and dogs were used. Complete sympathectomy was performed as described by Cannon, Newton, Bright, Menkin and Moore (1929). Dial (Ciba, 0.65 cc. per kilogram intraperitoneally), chloralose (0.1 gram per kilogram intravenously), urethane (1.0 to 1.5 gram per kilogram intravenously) or ether anesthesia was employed.

The blood pressure was recorded from a carotid or a femoral artery by means of a mercury manometer. A cannula was inserted into the trachea. The vagi were cut in the neck. The adrenal glands were ligated to eliminate a possible regrowth of their innervation.

The afferent nerves used were severed, and shielded electrodes were applied to their central ends. The stimuli were induction shocks from a Harvard induction coil with 5 volts in the primary circuit.

The respiration was frequently recorded, either by a Marey pneumograph or by connecting a recording tambour to one of the branches of a T-tube connected also with the tracheal cannula.

RESULTS. A. *Controls for regrowth of sympathetic fibers.* The denervated heart is very sensitive to adrenine (Anrep and Daly, 1925) and to sympathin (Cannon and Bacq, 1931). In animals which have been sympathectomized for some time (1 month or more) sympathetic preganglionic fibers may regrow to the adrenal glands (Bacq and Dworkin, 1930) and to the remaining sympathetic ganglia (prevertebral, sacral, cervical). Reflex activation of these fibers would lead to liberation of adrenine or sympathin which would be revealed by acceleration of the heart.

The experimental observations confirmed this regrowth when the animals were not studied shortly after the sympathectomy or when the two stages of the operation were separated by a long lapse of time. In such cases there was an acceleration of the heart attending certain afferent stimuli. This acceleration frequently persisted after adrenalectomy. In the cats it was usually abolished by evisceration or clamping the aorta at the diaphragm. In the dogs it was sometimes necessary to remove the inferior cervical ganglia to obtain an unvarying heart rate.

Only such observations as were made on animals whose heart rate was uniform were considered significant in the results to be reported.

B. *The anesthetics.* The negative results of clamping the carotids of sympathectomized cats under dial, which will be reported below (section C), led us to try the several anesthetics employed, for Bacq, Brouha and Heymans (*loc. cit.*) state that the low blood pressure common under dial is unfavorable for the appearance of carotid sinus reflexes.

The number of reliable experiments performed (see section A) and the anesthetics employed in them were as follows: dial—4 cats, 4 dogs; urethane—2 cats, 2 dogs; chloralose—2 cats, 2 dogs; ether—3 cats, 1 dog.

The experimental results were uniform under the various anesthetics. The responses to be described were, however, more marked when the blood pressure was higher. We may arrange the anesthetics, according to increasingly favorable effects on blood pressure, as follows: chloralose, ether, dial, urethane. The last anesthetic furnished the highest basal blood pressures.

C. *Carotid sinus reflexes.* They were tested by clamping the intact carotid when the blood pressure was being recorded from the other; or by clamping both carotids, the blood pressure being recorded from a femoral artery; or, finally, by denervating one carotid sinus and comparing the effects of clamping the innervated artery with those of the denervated control.

The results of these tests were in all instances minimal. Figure 1 illustrates the most noticeable effect recorded in the cats. Almost always

when one carotid was denervated as a control, the records from clamping this artery were indistinguishable from those obtained from the innervated side.

The blood pressure (cf. Bacq, Brouha and Heymans, *loc. cit.*) was quite satisfactory in the experiments; in no case was it lower than 80 mm. Hg, and it frequently was higher than 130 mm. Hg, especially under urethane.

It is noteworthy that in some of the animals which were discarded because the heart rate presented reflex variations, although probably only slight sympathetic functional regrowths were present after the previous

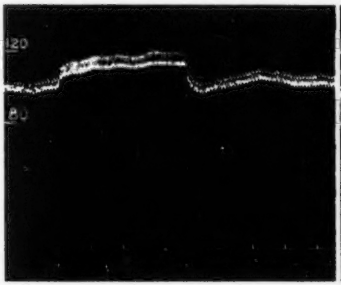


Fig. 1

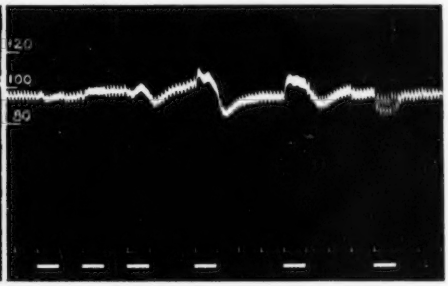


Fig. 2

Fig. 1. Completely sympathectomized cat. Chloralose. Vagi and depressors cut. Blood pressure recorded from the right carotid artery. Between the lower signals the left carotid was clamped. Time signal: 30-second intervals.

Fig. 2. Completely sympathectomized cat. Urethane. Vagi and depressors cut. Adrenals ligated. Eviscerated. The first five signals denote central stimulations of the left vagus and depressor with the following coil distances: 12, 11, 9, 7 and 5 cm. The last signal indicates occlusion of the tracheal cannula. Time signal: 30-second intervals.

complete sympathectomy, carotid sinus reflexes were readily detected with the technique described.

D. *Stimulation of afferent nerves.* We confirmed the previous report from this Laboratory (Freeman and Rosenblueth, *loc. cit.*) that stimulation of afferent nerves leads to changes of blood pressure in sympathectomized and vagotomized cats. We found further that dogs do not differ from cats in this respect.

Afferent stimulation of the left vagus and depressor nerves in the cats elicited immediate or delayed falls of blood pressure, or slight rises, sometimes succeeded by falls during stimulation. In all cases there were no movements other than those of respiration. The changes of blood pressure were not correlated with the respiratory effects. Thus, falls occurred when

the respiration was either accelerated or slowed. Figures 2 and 3 illustrate typical examples.

In the dogs weak afferent stimulation of the left vagus and depressor elicited a fall of blood pressure (fig. 4), strong stimulation sometimes in-

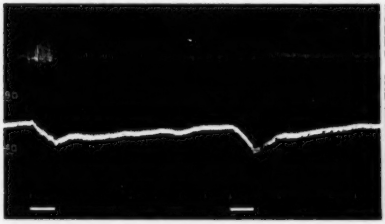


Fig. 3

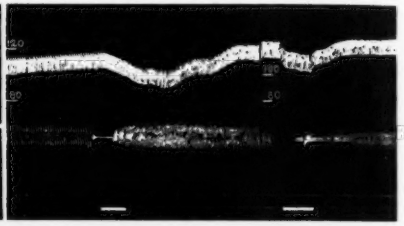
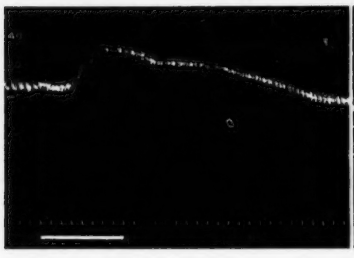


FIG. 4

Fig. 3. Completely sympathectomized cat. Dial. Vagi and depressors cut. Adrenals ligated. Central stimulations of the left vagus and depressor nerves; coil distances: 10 and 7 cm. Time signal: 30-second intervals.

Fig. 4. Completely sympathectomized dog. Urethane. Vagi and depressors cut. Central stimulation of the left vagus and depressor nerves; coil distances: 10 and 6 cm. Time signal: 30-second intervals.



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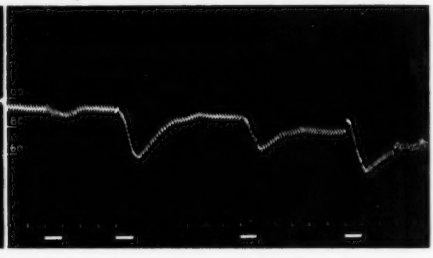

Fig. 6

Fig. 5. Completely sympathectomized dog. Urethane. Vagi and depressors cut. Central stimulation of the left vagus and depressor nerves; coil distance: 6 cm. Time signal: 5-second intervals.

Fig. 6. Completely sympathectomized cat. Urethane. Vagi and depressors cut. Adrenals ligated. Eviscerated. Central stimulations of a brachial nerve as follows: *1*, single shocks, 4 per second, 12 cm.; *2*, single shocks, 4 per second, 8 cm.; *3*, tetanizing, 8 cm.; *4*, tetanizing, 6 cm. Time signal: 30-second intervals.

duced rises (fig. 5). The respiration was usually inhibited with both the weak and the strong stimuli.

Central stimulation of the sciatic, the saphenous or a brachial nerve (somatic afferents) led to accelerated respiration in cats and dogs. Single shocks applied at frequencies of about two pairs per second evoked falls of

blood pressure. Weak shocks at tetanizing frequency likewise resulted usually in falls of blood pressure. Strong shocks at tetanizing frequency in the cats usually evoked a fall, exceptionally a rise; in the dogs usually a rise, or a fall succeeded by a rise, exceptionally only a fall of blood pressure. Consistently in cats or dogs, when strong tetanizing stimuli failed to yield a rise, the falls were smaller with these strong intensities than with weaker ones. Figures 6 and 7 illustrate these results.

Stimulation of the somatic afferents usually elicited spinal reflexes. These reflex movements were eliminated in some cases by section of the efferent nerves involved. Such a section altered slightly the magnitude, but not the sign of the blood-pressure changes.

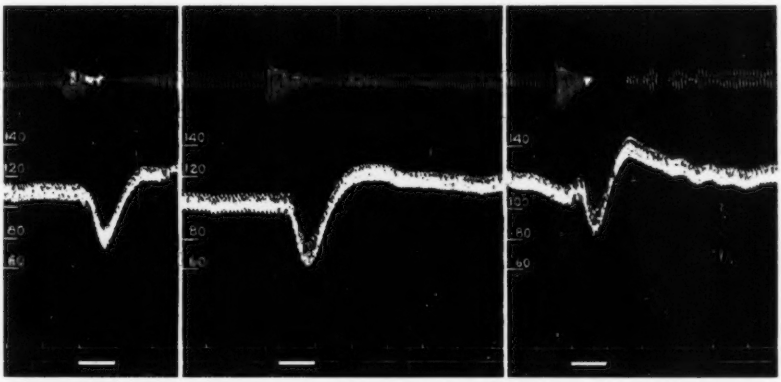

Fig. 7. Completely sympathectomized dog. Urethane. Vagi and depressors cut. Central stimulations of the saphenous nerve; coil distances: 12, 10 and 6 cm. Time signal: 30-second intervals.

E. *Asphyxia.* We confirmed the report of Bacq, Brouha and Heymans (*loc. cit.*) that inhalation of CO_2 results in prompt and marked falls of blood pressure in sympathectomized cats and dogs. These falls still occurred when curare and artificial respiration were employed to eliminate the increased respiratory movements which would otherwise ensue (cf. Rosenblueth and Cannon, *loc. cit.*).

Occlusion of the tracheal cannula for 30 seconds to 1 minute, on the other hand, led to augmented respiratory movements, but did not usually modify the blood-pressure level in either cats or dogs (cf. fig. 2). Exceptionally this occlusion produced a delayed slight fall of blood pressure.

F. *The splanchnic area.* Neither evisceration, nor ligation of the celiac and the superior and inferior mesenteric arteries, nor clamping the aorta at the diaphragm, all of which procedures eliminated the splanchnic vas-

cular area, modified the changes of blood pressure on afferent stimulation of nerves described in section E. Figures 2, 6 and 8 are illustrative of typical instances.

G. *Struggle.* Completely sympathectomized cats frequently faint when exerting any violent effort. This is in keeping with the fact that struggle is attended by a marked fall of blood pressure, whether the vagi are intact (Freeman and Rosenblueth, *loc. cit.*) or severed (Bacq, Brouha and Heymans, *loc. cit.*).

On the other hand, completely sympathectomized dogs, in our experience, may exert strong efforts (jumping, running, etc.) without failing as cats do. This difference between the two species has also been emphasized

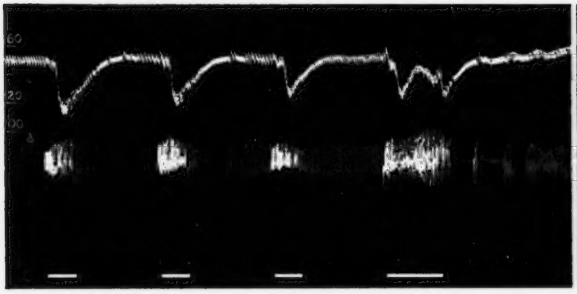

Fig. 8

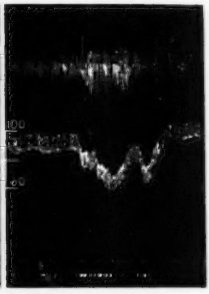

Fig. 9

Fig. 8. Completely sympathectomized dog. Dial. Vagi and depressors cut. Adrenals ligated. Aorta clamped at diaphragm. Central stimulations of a brachial nerve; coil distances: 12, 10, 8 and 6 cm.

Fig. 9. Completely sympathectomized dog. Ether. Vagi and depressors cut. The signals denote struggle on varying the depth of anesthesia.

by Bacq, Brouha and Heymans (*loc. cit.*). The question therefore arose whether the dog's blood pressure falls during struggle.

This question was studied in three dogs. In one, the spinal cord was cut aseptically at T8 and on the following day the blood pressure was recorded from a femoral artery without anesthesia. The vagi were intact in this animal. The other two dogs were anesthetized with ether, and the effects on the blood pressure of the struggle which attended varying the depth of anesthesia were recorded, first with the vagi intact, and later after section of these nerves in the neck.

The experimental results confirmed the direct observations of the behavior of the animals. Severe struggle produced only a slight fall of blood pressure (fig. 9) in striking contrast with the marked prompt falls which occur in the cats.

Discussion. I. *Carotid sinus reflexes.* Our negative results (section C)

confirm the similar entirely negative experience of Thomas and Brooks (1935). We realize that, under similar experimental conditions, negative evidence is of little significance when opposed to positive data such as those reported by Bacq, Brouha and Heymans for cats (*loc. cit.*, fig. 1A). Since these authors, however, do not mention any controls for regrowth of nerves to the adrenals and do not mention having ligated these glands, the question arises whether neglect of these controls may not have accounted for their positive experience. This suggestion seems plausible for the following reasons. From comparison of their figures 1A and 3 (the first illustrating a rise of blood pressure on occlusion of the carotids, the second a fall on stimulation of the carotid sinus nerve), it is obvious that, whether the vasomotor responses remaining after sympathectomy should be attributed to dilator nerves (Freeman and Rosenblueth, *loc. cit.*) or to constrictor nerves (Bacq, Brouha and Heymans), one of these two records denotes an inhibition of tone. This tone would be very high indeed to account for the large responses which they recorded. Their interpretation is quite inconsistent with the fact that section of the spinal cord, which would abolish this tone, does not produce any significant changes of blood pressure in sympathectomized cats (Rosenblueth and Cannon, *loc. cit.*).

The interpretation we have adopted, that the vasomotor nerves remaining after sympathectomy are the dorsal root dilators, leads us to expect that reflex rises of blood pressure can only be marked when the tone of this dilator system would be high. Reflex falls of blood pressure will, on the other hand, be easily obtained, particularly when the dilator tone is low. These expectations are confirmed by the data reported here, for occlusion of the carotids failed to induce any significant rise, while stimulation of the depressor fibers in the vagus caused marked falls of blood pressure (figs. 2, 3 and 4).

II. *Asphyxia and muscular metabolites.* The falls of blood pressure recorded on stimulation of the vagus were not due to anoxemia when the respiration was inhibited (figs. 3 and 4), because occlusion of the tracheal cannula for the same or a longer time than the cessation of respiration produced by the vagus did not induce a similar fall of blood pressure (fig. 2).

It is probable that the falls associated with increased respiratory activity (figs. 6, 7 and 8) were not due to an increased production of muscular metabolites, since on clamping the tracheal cannula increased respiratory activity did not lead to a similar fall of blood pressure. Reflex muscular activity was more pronounced with stronger than with weaker stimuli, yet the strong stimuli elicited less marked falls of blood pressure, or even rises (section D).

The reflex rises of blood pressure (figs. 2, 5 and 7) could only be due to reflex activation of constrictors or inhibition of dilator nerves.

The fall of blood pressure attending struggle of sympathectomized cats (section G) is probably not exclusively due to the increased production of muscular metabolites, as claimed by Bacq, Broucha and Heymans (*loc. cit.*). If muscular metabolites alone were at play, without any vasomotor nerve participation, we should expect the muscular metabolites of sympathectomized dogs to occasion a similar prompt and marked fall of blood pressure when these animals exert intense muscular activity. The effects of struggle on the blood pressure of dogs, however, are only slight (section G, fig. 9). This difference between dogs and cats we attribute to a greater activation of vasodilator nerves in the latter than in the former.

III. *The splanchnic area.* From the data presented (section F) we may conclude that the vascular reflexes of sympathectomized cats and dogs are not exclusively localized to this vascular area.

IV. *The nature of the non-sympathetic vasomotor nerves.* Bacq, Brouha and Heymans (*loc. cit.*) report a marked and prolonged fall of the blood pressure of sympathectomized cats when 1 mgm. percaine was injected into the spinal canal in the cervical region. This fall they attribute to a "physiological section" of the spinal cord by the drug. The disconnection of the circulatory system from higher centers would eliminate the tonic influence of these centers. Since the result was a fall of blood pressure, they conclude that the non-sympathetic vasomotor fibers in the cat are constrictor nerves. Percaine may, however, have exerted other effects in these experiments than the hypothetical paralysis of vasomotor paths. Indeed, when we consider that true section of the spinal cord in the cervical region does not modify the blood pressure of sympathectomized cats (Rosenblueth and Cannon, *loc. cit.*), we feel justified in dismissing the evidence from percaine as inconclusive.

Since section of the spinal cord does not induce changes of blood pressure in the sympathectomized cats, it follows that the remaining vasomotor system in these animals does not receive a significant tonic activation from the higher centers. Inhibition of this vasomotor system will therefore be difficult to demonstrate. We find (figs. 3 and 6) that falls of blood pressure are readily obtained in these cats, while rises are seldom demonstrable (section D). This evidence is in accord with the view that the non-sympathetic vasomotor system of the cat is a vasodilator, not a vasoconstrictor system.

Atropine does not abolish the vasomotor reflexes of sympathectomized cats (Bacq, Brouha and Heymans, *loc. cit.*) while curare does abolish them (Freeman and Rosenblueth, *loc. cit.*). This evidence is in accord with the suggestion previously made (see introduction), that the vasodilator fibers in question are the dorsal root dilators.

V. *The differences between sympathectomized cats and dogs.* In one

respect the two species were found to differ. Struggle in cats induces more severe falls of blood pressure than is the case in dogs (section G). Whether this difference is not merely quantitative remains for future experiments to decide.

SUMMARY

The blood-pressure reactions to afferent stimulation of several nerves, to occlusion of the carotids, to asphyxia, and to struggle were studied in completely sympathectomized and vagotomized cats and dogs.

Reflex rises and falls of blood pressure were obtained in the two species on stimulation of afferent nerves (figs. 3, 4, 5 and 7). Exclusion of the splanchnic vascular area did not abolish these responses (figs. 2, 6 and 8).

No significant reflex rises of blood pressure were obtained on occlusion of the innervated carotids (fig. 1, section C) in either cats or dogs.

Struggle is attended by a sharp severe fall of blood pressure in cats but not in dogs (fig. 9, section G). This was the only difference detected between the two species.

The mechanism of these reactions is discussed. It is shown that they are at least partly controlled by non-sympathetic vasomotor nerves (p. 718) and that these nerves are probably the dorsal root dilators (p. 719).

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